Tulane University
Human Research Protection Program

Tulane Human Research Protection Program
Institutional Review Boards
Biomedical
Social Behavioral
FWA00002055

DATE:	December 17, 2014
TO:	James Robinson, M.D. and Robert F. Garry, Ph.D.
FROM:	Tulane University Biomedical IRB
STUDY TITLE:	[140674-16] Roles of Protective or Pathogenic B Cell Epitopes in Human Lassa Fever and Ebola Virus Disease (09-00419)
IRB REFERENCE #:	09-00419
SUBMISSION TYPE:	Amendment/Modification
ACTION:	APPROVED
EFFECTIVE DATE:	**December 16, 2014**
EXPIRATION DATE:	**June 11, 2015**
REVIEW TYPE:	Expedited Review
PROJECT RISK LEVEL:	Minimal Risk

Thank you for submitting the Amendment/Modification for the above referenced study. The Tulane IRB approved your submission including:

- Amendment/Modification - B Cell_Amendment_Dec 2014 (UPDATED: 12/11/2014)
- Application for Human Subjects Research, Part 1 - Application for Human Subjects Research, Part 1 (UPDATED: 12/11/2014)
- Child Assent - B Cell Assent_SL_track changes (UPDATED: 12/16/2014)
- Child Assent - B Cell Assent_SL_clean (UPDATED: 12/16/2014)
- Child Assent - B Cel_Assent SCript_SL (UPDATED: 12/16/2014)
- Consent Form - B Cell Consent_SL_track changes (UPDATED: 12/16/2014)
- Consent Form - B Cell _Consent_SL_clean (UPDATED: 12/16/2014)
- Consent Form - B Cell_Consent Script_SL (UPDATED: 12/16/2014)
- Cover Sheet - B Cell_Cver Sheet_Dec 2014 (UPDATED: 12/16/2014)
- Other - Emergency Contact Cards (UPDATED: 12/12/2014)
- Other - ICIDR Grant Application (UPDATED: 12/11/2014)
- Protocol - B Cell_Protocol_Dec2014_clean (UPDATED: 12/12/2014)
- Protocol - B Cell_Protocol_Dec2014_track changes (UPDATED: 12/12/2014)

The Tulane University Biomedical IRB has conducted an expedited review and has granted a CONDITIONAL APPROVAL of an amendment to the research titled, "Roles of Protective or Pathogenic B Cell Epitopes in Human Lassa Fever and Ebola Virus Disease," in accordance with 45 CFR 46.110(b)(1). The amendment to the protocol includes:

1. The addition of a new funding source from NIH;

2. A revision to the consent process to allow for obtaining verbal consent, using a consent script, to support strict infection control practice, due to the circulation of Ebola Virus in Sierra Leone;

3. Revisions to the protocol to address requirements from the new funding source. Serology analysis and auditory evaluation for hearing loss are now included in the protocol, as well as the revised consent/assent documents and scripts.

Please provide the Tulane University Biomedical IRB with the approval letter from the Sierra Leone Ethics and Scientific Research Committee, prior to implementing the amended procedures.

Criteria for approval continues in accordance with 45 CFR 46.111(a)(1-7). Children may be enrolled in research not involving greater than minimal risk in accordance with §46.404. The permission by parents or guardians and for assent by children in accordance with §46.408.

This study is approved for the enrollment of 5,300 patients; 2,500 from Nigeria and 2,800 from Sierra Leone which includes 300 Ebola patients. The Investigator reports that 2,355 patients have been enrolled.

IRB approval for this study will expire June 11, 2015.

This approved protocol is supported by NIH grant HHSN272200900049C (Roles of protective or pathogenic B cell epitopes in human Lassa fever) with funding pending under grant opportunity 1 U19 AI115589-01 (International Collaboration in Infectious Disease Research on Lassa Fever and Ebola).

The most recent IRB approved and stamped informed consent/assent form(s) are to be used when enrolling subjects.

If there are any pending approvals from any other institutions or other research oversight committees, the research cannot commence until all such approvals have been obtained, and the PI is to provide to the Tulane IRB via IRBNet a copy of all approval letters as received. This includes Tulane Institutional Biosafety approval (when applicable), Tulane Radiation Safety Committee approval (when applicable), and any other committee approval required by the University. Additionally, for sponsored research, the research cannot commence until the sponsored research agreement has been fully executed.

If you have any questions, please contact the HRPO at (504) 988-2665 or irbmain@tulane.edu.

Sincerely,
Tulane University Human Research Protections Office
1440 Canal St, Suite 1705, TW-36
New Orleans, LA 70112

Please note that the actual signature by the IRB Chair(s) is not required for this document to be effective since it is generated by IRBNet pursuant to the IRB Chair's electronic signature and approval. This process is consistent with Federal Regulations and Tulane standard operating policies with respect to the IRB and Human Research Protection Office, which consider electronically generated documents as official notice to sponsors and others of approval, disapproval or other IRB decisions. Please refer to the HRPO website at http://tulane.edu/asvpr/irb to refer to Tulane's Electronic Signatures and Records Policy.

GOVERNMENT OF SIERRA LEONE
Office of the Sierra Leone Ethics and Scientific Review Committee
Directorate of Training, Non-Communicable Diseases and Research
Connaught Hospital
Ministry of Health and Sanitation

2nd December, 2014

To: Dr. John S. Schieffelin
Departments of Pediatrics and Internal Medicine
Tulane University School of Medicine
1430 Tulane Avenue TB - 8
New Orleans, Louisiana 70112

Study Title: Development of a Recombinant Antigen Diagnostic for Ebola Zaire Antigen Detection
Committee Action: Expedited Review
Approval Date: 2nd December, 2014
Submission Type: Initial Protocol Approval Version 1.0 of 9th November, 2014

The Sierra Leone Ethics and Scientific Review Committee (SLESRC) having conducted an expedited review of the above study protocol and determined that this protocol presents minimal risk to subjects, hereby grants ethical and scientific approval for the study to be conducted in Sierra Leone. The approval is valid for the period, 2nd December 2014 – 1st December, 2015. It is the responsibility of an investigator to obtain re-approval for any ongoing research prior to its expiration date. The request for re-approval must be supported by a progress report.

Review Comments:

- To use written informed consent with witness signature (for illiterate participants) for blood, saliva, breast milk and urine sample collections. Provide contact 076629251, for SLESRC Secretariat. Augustine Gobba's mobile non-responsive.
- Amendments: Intended changes to the approved protocol such as the informed consent documents, study design, recruitment of participants and key study personnel, must be submitted for approval by the SLESRC prior to implementation.
- Termination of the study: When study procedures and data analyses are fully complete, please inform the SLESRC that you are terminating the study and submit a brief report covering the protocol activities. Individual identifying information should be destroyed unless there is sufficient justification to retain, approved by the SLESRC. All findings should be based on de-identified aggregate data and all published results in aggregate or group form.

Professor Hector G. Morgan
Chair

Email: Personal Info

Tulane University
Human Research Protection Program

Tulane Human Research Protection Program
Institutional Review Boards
Biomedical
Social Behavioral
FWA00002055

DATE:	November 14, 2014
TO:	John Schieffelin, MD
FROM:	Tulane University Biomedical IRB
STUDY TITLE:	[682862-1] Development of a Recombinant Antigen Diagnostic for Ebola Zaire Antigen Detection
IRB REFERENCE #:	14-682862
SUBMISSION TYPE:	New Project
ACTION:	APPROVED WITH CONDITIONS
APPROVAL DATE:	**November 14, 2014**
EXPIRATION DATE:	**November 13, 2015**
REVIEW TYPE:	Expedited Review
PROJECT RISK LEVEL:	Minimal Risk

Thank you for your recent initial submission. The Tulane University Institutional Review Board has approved your submission.

This approval is based on an appropriate risk/benefit ratio and a study design where the risks have been minimized. All research must be conducted in accordance with this approved submission.

The following items were included in this submission:

- Application for Human Subjects Research, Part 1 - Application for Human Subjects Research, Part 1 (UPDATED: 11/13/2014)
- Application Form - ReEBOV Application part 2 (UPDATED: 11/13/2014)
- Consent Form - EBOV RDT Consent Script (UPDATED: 11/11/2014)
- Cover Sheet - Cover SHeet_EBOV RDT_Initial (UPDATED: 11/11/2014)
- CV/Resume - Schieffelin_CV (UPDATED: 11/11/2014)
- Other - Information Card (UPDATED: 11/13/2014)
- Other - routing sheet (UPDATED: 11/11/2014)
- Other - Package Insert (UPDATED: 11/11/2014)
- Proposal - R44 (UPDATED: 11/11/2014)
- Protocol - ReEBOV Protocol_v1 (UPDATED: 11/13/2014)

- Training/Certification - Ethics Training - Lee (UPDATED: 11/11/2014)
- Training/Certification - Ethics Training - Mustapha (UPDATED: 11/11/2014)
- Training/Certification - Ethics Training - Koroma (UPDATED: 11/11/2014)
- Training/Certification - Ethics Training - Kamara (UPDATED: 11/11/2014)
- Training/Certification - Ethics Training_Kanneh (UPDATED: 11/11/2014)
- Training/Certification - Ethics Training_Gbackie (UPDATED: 11/11/2014)
- Training/Certification - Ethics Training_Goba (UPDATED: 11/11/2014)
- Training/Certification - Ethics Training_Grant (UPDATED: 11/11/2014)
- Training/Certification - CITI_Boisen (UPDATED: 11/11/2014)
- Training/Certification - CITI_Garry (UPDATED: 11/11/2014)
- Training/Certification - CITI_Moses (UPDATED: 11/11/2014)

Tulane University Biomedical IRB has provided an expedited IRB review and has granted a CONDITIONAL APPROVAL of the above referenced study in accordance with federal regulation 45 CFR 46.110(b)(1), research category 2, 3, and 5.

1. Approval from the Sierra Leone Ethics and Scientific Review Committee must be obtained and provided to the Tulane University Biomedical IRB for review and acknowledgment prior to conducting any study procedures.

2. Per Genean Mathieu, Administrative Compliance Specialist for the Tulane University Conflict of Interest Committee, there is an outstanding COI matter. Co-Investigator Sylvia Lee is to complete all parts of the COI Disclosure Form and CITI COI Training. Co-Investigator Lee may not participate in research activities until this matter is addressed. Please contact Ms. Mathieu at coi@tulane.edu or (504) 247-1286 for guidance and/or clarification.

Criteria for IRB approval of research is in accordance with 45 CFR 46.111(a)(1-7). A waiver for the documentation of consent is granted in accordance with 45 CFR 46.117()(2). For the purpose of using the de-identified excess blood sample, after qRT-PCR testing is performed, a waiver of consent is appropriate in accordance with 45 CR 46.116(d)(1-4).

Pregnant women may participate in this minimal risk study in accordance with 45 CFR 46.204 and 205. Children may participate in this minimal risk study in accordance with 45 CFR 46.404. Adequate provisions have been made for soliciting the assent of the children and permission of their parents or guardians, as set forth in §46.408.

The IRB has approved the enrollment of 500 subjects for blood/specimen collection and 1,000 de-identified blood samples. This study is granted an approval period of November 14, 2014 - November 13, 2015.

Please Note:

- The Tulane University IRB approved and stamped consent/assent documents are to be utilized when enrolling subjects.

Proposed changes to the research must be submitted to the IRB for review and approved prior to implementation, unless a change is necessary to avoid immediate harm to subjects.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of their signed consent form unless this requirement has been waived by the IRB.

Any Unanticipated Problems involving Risk to Subjects or Others, Deviations from the approved research, Non-Compliance, and Complaints must be reported to the IRB in accordance with Tulane HRPP policies and procedures. If this study includes ongoing oversight by a Data Safety Monitoring Board (DSMB) or other such committee, reports generated by the DSMB or oversight committee must be submitted to the IRB.

Continuations must be submitted in accordance with Tulane HRPP policies and procedures. The federal regulations provide for no grace period. Failure to obtain approval for continuation of your study prior to the expiration date will require discontinuation of all research activities for this study, including enrollment of new subjects.

When all study activities and data analysis have been completed, please notify the IRB within 30 days by submitting a Study Closure Form.

If you have any questions regarding this approval, please contact the HRPO at (504) 988-2665 or irbmain@tulane.edu.

Sincerely,
Tulane University Human Research Protections Office
1440 Canal St, Suite 1705, TW-36
New Orleans, LA 70112

Please note that the actual signature by the IRB Chair(s) is not required for this document to be effective since it is generated by IRBNet pursuant to the IRB Chair's electronic signature and approval. This process is consistent with Federal Regulations and Tulane standard operating policies with respect to the IRB and Human Research Protection Office, which consider electronically generated documents as official notice to sponsors and others of approval, disapproval or other IRB decisions. Please refer to the HRPO website at http://tulane.edu/asvpr/irb to refer to Tulane's Electronic Signatures and Records Policy.

GOVERNMENT OF SIERRA LEONE
Office of the Sierra Leone Ethics and Scientific Review Committee
Directorate of Training, Non-Communicable Diseases and Research
Connaught Hospital
Ministry of Health and Sanitation

9th October, 2014

Dr. John S. Schieffelin
Tulane University School of Medicine
Departments of Paediatrics and Internal Medicine
1430 Tulane Ave TB-8
New Orleans, LA 70112
USA

Dear Dr. Schieffelin,

EBOLA ANTIGEN RAPID DIAGNOSTIC TEST (EBOVAgRDT)

This letter is a response to yours of 2nd October, 2014 concerning the proposed rapid diagnostic test.

It is clearly understood that this is a RESEARCH STUDY restricted to and falls under the waiver of Informed Consent ii. in the protocol for Epidemiologic and Clinical Characterization of an Ebola Virus Outbreak, Sierra Leone approved by the SLESRC on 8th September, 2014.

We look forward to a comprehensive report of this comparative research study and presentation of any further activity you may wish to be considered by relevant National Regulatory Authorities including the SLESRC.

Yours sincerely,

Prof. Hector G. Morgan
Chair

Cc: Robert F. Garry Ph D
Pardis Sabeti MD Ph.D
Douglas Simpson CEO Corgenix Inc
Prof. S.M. Gevao
Augustine Goba, Director, Lassa Fever Laboratory

Email: Personal Info

TELEGRAMS..... TELEPHONE.....

MINISTRY OF HEALTH

DEPARTMENT OF PLANNING, RESEARCH & STATISTICS DIVISION

PRIVATE MAIL BAG NO. 5027, OYO STATE OF NIGERIA

Your Ref. No. .....

All communications should be addressed to

the Honorable Commissioner quoting

Our Ref. No. AD 13/ 479/____ October, 2014

The Principal Investigator,
Malaria Research Laboratories,
IMRAT,
College of Medicine,
University of Ibadan,
Ibadan, Nigeria.

Attention: Professor Christian Happi

Re: Ethical Approval for the Implementation of your Research Proposal in Oyo State

In response of your letter requesting for Renewal of your Research Proposal tittled: "Host Genetic Factors in Resistance to Lassa Hamorrhagic Fever."

2. The committee has noted your compliance with all the ethical concerns raised in the initial review of the proposal. In the light of this, I am pleased to convey to you the approval of committee for the implementation of the Research Proposal in Oyo State, Nigeria.

3. Please note that the committee will monitor closely and follow up the implementation of the research study. However, the Ministry of Health would like to have a copy of the results and conclusions of the findings as this will help in policy making in the health sector.

4. Wishing you all the best.

OYO STATE RESEARCH ETHICAL REVIEW COMMITTEE
Signature & Date

Sola Akande (Dr)
Director, Planning, Research & Statistics
Secretary, Oyo State, Research Ethical Review Committee

Tulane University
Human Research Protection Program

Tulane Human Research Protection Program
Institutional Review Boards
Biomedical
Social Behavioral
FWA00002055

DATE: July 30, 2014

TO: John Schieffelin, MD
FROM: Tulane University Biomedical IRB

STUDY TITLE: [631715-1] Epidemiologic and Clinical Characterization of an Ebola Outbreak, Sierra Leone, 2014
IRB REFERENCE #: 14-631715
SUBMISSION TYPE: New Project

ACTION: APPROVED

APPROVAL DATE: July 30, 2014

EXPIRATION DATE: July 29, 2015

REVIEW TYPE: Expedited Review
PROJECT RISK LEVEL: Minimal Risk

Thank you for your recent initial submission. The Tulane University Institutional Review Board has approved your submission.

This approval is based on an appropriate risk/benefit ratio and a study design where the risks have been minimized. All research must be conducted in accordance with this approved submission.

The following items were included in this submission:

- Application for Human Subjects Research, Part 1 - Application for Human Subjects Research, Part 1 (UPDATED: 07/9/2014)
- Application Form - Application Part 2_Ebola Outbreak (UPDATED: 07/27/2014)
- Cover Sheet - Cover Sheet_Initial Review (UPDATED: 07/9/2014)
- CV/Resume - CV_Schieffelin (UPDATED: 07/9/2014)
- Data Collection - KGH LFW Chart forms (UPDATED: 07/27/2014)
- Letter - Letter from SL MOHS (UPDATED: 07/9/2014)
- Other - Checklst_Ebola 2014 (UPDATED: 07/8/2014)
- Protocol - Ebola_protocol_v1 (UPDATED: 07/27/2014)
- Training/Certification - IRB training_Grant (UPDATED: 07/9/2014)
- Training/Certification - CITI Training_Garry (UPDATED: 07/9/2014)

- Training/Certification - IRB Training_Khan (UPDATED: 07/8/2014)
- Training/Certification - IRB Training_Goba (UPDATED: 07/8/2014)
- Training/Certification - IRB Training_Gbakie (UPDATED: 07/8/2014)
- Training/Certification - CITI Training_Moses (UPDATED: 07/8/2014)
- Training/Certification - CITI Training_Shaffer (UPDATED: 07/8/2014)

Tulane University Biomedical IRB has provided an expedited IRB review and approval of the above referenced study in accordance with federal regulation 45 CFR 46.110(b)(1), research category 4 and 5.

Criteria for IRB approval of research is in accordance with 45 CFR 46.111(a)(1-7). Data collected from children may be included in accordance with 45 CFR 46.404. The IRB grants a waiver of informed consent in accordance with 45 CFR 46.116(d)(1-4). A waiver for documentation of consent is granted in accordance with 45 CFR 46.117(c)(2).

This study is granted an approval period of July 30, 2014 - July 29, 2015.

Please Note:

- This research study has been approved for the enrollment of 1,000 charts . An Amendment must be submitted, reviewed, and approved before exceeding this amount.

Proposed changes to the research must be submitted to the IRB for review and approved prior to implementation, unless a change is necessary to avoid immediate harm to subjects.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of their signed consent form unless this requirement has been waived by the IRB.

Any Unanticipated Problems involving Risk to Subjects or Others, Deviations from the approved research, Non-Compliance, and Complaints must be reported to the IRB in accordance with Tulane HRPP policies and procedures. If this study includes ongoing oversight by a Data Safety Monitoring Board (DSMB) or other such committee, reports generated by the DSMB or oversight committee must be submitted to the IRB.

Continuations must be submitted in accordance with Tulane HRPP policies and procedures. The federal regulations provide for no grace period. Failure to obtain approval for continuation of your study prior to the expiration date will require discontinuation of all research activities for this study, including enrollment of new subjects.

When all study activities and data analysis have been completed, please notify the IRB within 30 days by submitting a Study Closure Form.

If you have any questions regarding this approval, please contact the HRPO at (504) 988-2665 or irbmain@tulane.edu.

Sincerely,
Tulane University Human Research Protections Office
1440 Canal St, Suite 1705, TW-36
New Orleans, LA 70112

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GOVERNMENT OF SIERRA LEONE
Office of the Sierra Leone Ethics and Scientific Review Committee
Directorate of Training, Non-Communicable Diseases and Research
Connaught Hospital
Ministry of Health and Sanitation

16th July, 2014

Dr. Sheik Humarr Khan
Kenema Government Hospital
Combema Road
Kenema

Dear Dr. Khan,

Emergency Response to the Ebola Virus Outbreak in Sierra Leone: Clinical and Epidemiological Data for De-Identified Samples

I refer to your request of 13th July, 2014 for approval to use clinical and epidemiological data for de-identified samples collected from all suspected EVD patients receiving care during this response.

The Ethics Committee hereby grants approval for this use and endorses the collection of samples without written informed consent, during this emergency.

Yours sincerely,

Professor Hector G. Morgan
Chair

Email: Personal Info

GOVERNMENT OF SIERRA LEONE
MINISTRY OF HEALTH AND SANITATION
OFFICE OF THE CHIEF MEDICAL OFFICER

18th June, 2014

Harvard University
52, Oxford Street,
NWL 469.3
Cambridge, MA 02138

Dear Prof. Sabeti

RE: EBOLA VIRUS SEQUENCING FROM SIERRA LEONE

The purpose of this letter is to permit a waiver of consent to sequence and make publicly available viral sequences obtained from patient and contact samples collected during the current EBOLA outbreak in Sierra Leone.

Researchers at Harvard University and Broad institute, with partners at Tulane University, should sequence samples from suspected EBOLA virus infected individuals and make viral sequences publicly, available, through both the NIH's National Centre for Biotechnology Information database and open-access journal publication. The information from these sequences will help researchers tract the spread of the virus, as well as inform the development of more sensitive diagnostics for use by the scientific community. It is imperative that these sequences be published as quickly as possible so that these benefits can be implemented rapidly and effectively in this outbreak setting.

Due to the unique circumstances surrounding this EBOLA outbreak, we are granting a waiver of consent to study these virus-positive EBOLA samples collected from suspected EBOLA cases and contacts. This approval covers the sequencing of viral genomes contained in these samples, with no analysis conducted on human genetic material.

Patient involvement in this research carries minimal or no risk to participants. These are diagnostic samples taken in the course of clinical care and infection surveillance. Researchers should analyse only viral genetic data from the samples and will discard any data pertaining to host genomes.

MINISTRY OF HEALTH AND SANITATION, 4th Floor Youyi Building, Brookfields, Freetown
Contact Mobile No: Personal Info ; Email: Personal Info

There is an established plan to protect patient privacy and data confidentiality: the samples will be sent from Sierra Leone to the United States in a de-identified manner. All patient identifying information should be stripped from each sample, and each sample given a unique, randomly-generated Sample Identification Number. The only data associated with these samples should be non-identifying clinical and demographic data, such as symptoms, outcome of disease, or large-scale geographic information.

Yours sincerely,

Dr. Brima Kargbo (GCOR)
Chief Medical Officer

College of Medicine & Allied Health Sciences
University of Sierra Leone

PROFESSOR SAHR MOSES GEVAO
MBBS, FWACP, FIAC (PATH I) (Nig) MISH
HEAD: DEPT. OF HAEMATOLOGY

NEW ENGLAND
FREETOWN
SIERRA LEONE
Mobile: Personal Info
Personal Info

REFERENCES

Date 30th May, 2014

The Chairman
Sierra Leone Ethics and Scientific Review Committee
Freetown

THROUGH: The Chief Medical Officer, MoHS 18/6/14
Youyi Building
Freetown

Dear Sir,

Shipment of Non – Infectious Non Biological Samples

I write to inform you that the Ministry of Health and Sanitation has approved that non infectious non biological samples from Kenema Viral Haemorrgic fever laboratory be shipped to Broad Institute, Havard University, USA for genomic studies.

Please find below necessary information regarding the samples.

Shipper	-	Dr. H. Kahn
Status of samples	-	Non Infectious
Recipient	-	Dr. Pardin Sabeti
Address	-	Havard University, 52 Oxford Street, RM 469, Cambridge, MA, 02138. Phone No Personal Info

Kind Regards,

Prof. (Dr.) S.M. Gevao
Viral Haemorrgic fever
Consortium

HARVARD
Human Research Protection Program

Harvard University-Area
Committee on the Use of Human Subjects
1414 Massachusetts Avenue, 2nd Floor
Cambridge, MA 02138
Federal Wide Assurance FWA00004837

Notification of Continuing Review Approval

April 21, 2014

Pardis Sabeti
pardis@broadinstitute.org

Protocol Title:	Human and microbial genetic factors important in Lassa fever
Protocol #:	CR-22362-01
Funding Source:	The Broad Institute, Tulane University, Office of the Director, NIH
IRB Review Date:	4/21/2014
Effective Date:	4/21/2014
Expiration Date:	5/16/2015
IRB Review Type:	Expedited
IRB Review Action:	Approved

Dear Pardis Sabeti:

On 4/21/2014, after review of your Continuing Review, the Institutional Review Board (IRB) of the Harvard University-Area has approved the above-referenced submission. **Please note that the approval for this protocol will lapse on 5/16/2015.**

This approval includes the following:
- CC NG Assent (exp2014).pdf (Consent Materials) version: 2013
- CC NG Consent (exp2014).pdf (Consent Materials) version: 2013
- Case Control in Nigeria (Protocol Documents) version: 2013.10.24
- Modification 1 (Protocol Documents) version: 3/2013
- Renewal 2013 (Protocol Documents) version: 4/2013
- F22362 Current Protocol (2013.12.20).docx (Protocol Documents) version: 12/20/2013

Additionally, the IRB has reviewed the following documents:
- Terms & Conditions (Data Use Agreement or Other Agreements) version: 2013.11.15
- Tulane Renewal Approval (External Site Information) version: 3/11/13
- ISTHREC Renewal Approval (External Site Information) version: 5/7/13
- UTMB Exempt Approval (External Site Information) version: 11/10/13

The IRB made the following determinations:
- Special Populations: Children

- Waivers: None
- Risk Determination: No greater than minimal risk
- Research Information Security Level: The research is classified, using Harvard's Data Security Policy, as Level 1 Data.

Please contact me at 617-496-1833 or amaislen@fas.harvard.edu if you have any questions.

Sincerely,

Andrea Maislen
Research Officer

GOVERNMENT OF SIERRA LEONE
Office of the Sierra Leone Ethics and Scientific Review Committee
Directorate of Training, Non-Communicable Diseases and Research
Connaught Hospital
Ministry of Health and Sanitation

16th January, 2014

Professor Robert F. Garry
Department of Microbiology and Immunology School of Medicine
Tulane University Health Sciences Center
1430 Tulane Avenue
SL – 38, New Orleans, LA 70112 - 2699

Dear Professor Garry,

Host Genetic Factors in Resistance to Lassa Hemorrhagic Fever

Reference your letter of 12th December, 2013 concerning change of the laboratory where all genetic analysis will be carried out.

The Committee accepts your replacement of the Broad Institute and Harvard University with Bina Technologies and the assurance of continued safeguard against disclosure of data to unauthorised persons

The Committee hereby grants renewal of approval for the amended study for the period 16th January, 2014 to 15th January, 2015.

The Committee stipulates as follows: that,

1. It must be notified in advance, if you decide to further amend the approved research design and/or methodology at any time during the conduct of the study.
2. It must be informed if for any reason, the study is terminated prematurely.
3. On the conclusion of the study, you submit a report or any publication based on the study.

Yours sincerely,

Professor Hector G. Morgan
Chairman, SLESRC

Email: Personal Info

HARVARD
Human Research Protection Program

Harvard University-Area
Committee on the Use of Human Subjects
1414 Massachusetts Avenue, 2nd Floor
Cambridge, MA 02138
Federal Wide Assurance FWA00004837

Notification of Continuing Review Approval

October 29, 2013

Pardis Sabeti
pardis@broadinstitute.org

Protocol Title:	Human and microbial genetic factors important in Lassa Fever - Sierra Leone
Protocol #:	CR-21288-01
Funding Source:	The Broad Institute, Office of the Director, NIH
IRB Review Date:	10/29/2013
Effective Date:	11/19/2013
Expiration Date:	11/18/2014
IRB Review Type:	Expedited
IRB Review Action:	Approved

Dear Pardis Sabeti:

On 10/29/2013, after review of your Continuing Review, the Institutional Review Board (IRB) of the Harvard University-Area has approved the above-referenced submission. **Please note that the approval for this protocol will lapse on 11/18/2014.**

This approval includes the following:
- F21288 Current Protocol (2013.10.24).doc (Protocol Documents) version: 0.01
- SL Assent (Consent Materials) version: Exp2014
- SL Consent (Consent Materials) version: Exp2014

Additionally, the IRB has reviewed the following documents:
- Cede Review Approval (External Site Information) version: 7/2013
- Tulane Renewal Approval 2013 (External Site Information) version: 0.01
- SLESRC 2012 Renewal Approval (External Site Information) version: 0.01

The IRB made the following determinations:

- Special Populations: Children, Pregnant Women
- Waivers: None
- Risk Determination: No greater than minimal risk

- Research Information Security Level: The research is classified, using Harvard's Data Security Policy, as Level 1 Data.

If you have any questions, please contact me at 617-496-8301 or john_ennever@harvard.edu

Sincerely,

John Ennever
Director of IRB Policy Development and Compliance

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Freedom of Information Office
5601 Fishers Lane, Suite 6G50
Bethesda, Maryland 20892
Tel (301) 451-5109
Fax (301) 480-0904/ Email foia@niaid.nih.gov

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, MD 20892

March 27, 2019

Emanuel Freudenthal
MuckRock News
DEPT MR 68065
411A Highland Avenue
Somerville, MA 02144-2516

Re: FOI Case No. 50124

Dear Mr. Freudenthal:

This is a final response to your January 24, 2019 Freedom of Information Act (FOIA) request emailed to the National Institute of Allergy and Infectious Diseases (NIAID) FOIA Office, which was received in this office that same day. In two separate emails you requested the following:

"1 - Contracts and the latest report for the following projects:
- 1R13AI104216-01 (http://grantome.com/grant/NIH/R13-AI104216-01);
- 1U19AI115589-01 (http://grantome.com/grant/NIH/U19-AI115589-01);
- 5U19AI115589-02 (http://grantome.com/grant/NIH/U19-AI115589-02)

2 - Any list of people receiving payment as part of these projects. If such a list does not exist, then I'd like all of the contracts with people paid under these projects.

3 - Any/all agreements (such as contracts, Memorandum of Understanding, Terms of Reference, Material Transfer Agreements etc) of NIAID with the Kenema hospital from 01/01/2013 to 01/01/2017.

4 - Any/all agreements (such as contracts, Memorandum of Understanding, Terms of Reference, Material Transfer Agreements etc) of Tulane University with the Kenema hospital 01/01/2013 to 01/01/2017.

5 - Any/all reports or evaluation of the effectiveness/reliability of Ebola tests/assays by members of the Viral Hemorrhagic Fever Consortium (i.e. Autoimmune Technologies, Broad Institute of MIT and Harvard University, Corgenix, The University of Texas Medical Branch at Galveston (UTMB), The Scripps Research Institute, the University of California at San Diego, the Irrua Specialist Teaching Hospital Lassa Fever Program, and the Kenema Government Hospital).

PS: please include the following to the list of contracts in #1 above
- 1U01AI082119-03 (http://grantome.com/grant/NIH/U01-AI082119-01
- HHSN272200900049C

And add:
6 - The Institutional Review Board approvals related to Ebola sample collection or data collect from 01/01/2014 with either Robert F. Garry or John Schieffelin as Principal Investigators"

Your requests were broken down into seven separate requests and assigned the following FOIA Case Numbers:

FOIA Case No. 50120 - Contracts and the latest report for the following projects:
- 1R13AI104216-01 (http://grantome.com/grant/NIH/R13-AI104216-01);
2 - Any list of people receiving payment as part of these projects. If such a list does not exist, then I'd like all of the contracts with people paid under these projects.

FOIA Case No. 50121 - Contracts and the latest report for the following projects:
1U19AI115589-01 (which will include 5U19AI115589-02)
(http://grantome.com/grant/NIH/U19-AI115589-01);
2 - Any list of people receiving payment as part of these projects. If such a list does not exist, then I'd like all of the contracts with people paid under these projects.

FOIA Case No. 50122 - Contracts and the latest report for the following projects -
1U01AI082119-03 (http://grantome.com/grant/NIH/U01-AI082119-01
2 - Any list of people receiving payment as part of these projects. If such a list does not exist, then I'd like all of the contracts with people paid under these projects.

FOIA Case No. 50123 - please include the following to the list of contracts in #1 above
HHSN272200900049C

FOIA Case No. 50124 - Any/all agreements (such as contracts, Memorandum of Understanding, Terms of Reference, Material Transfer Agreements etc) of NIAID with the Kenema hospital from 01/01/2013 to 01/01/2017. Any/all agreements (such as contracts, Memorandum of Understanding, Terms of Reference, Material Transfer Agreements etc.) of Tulane University with the Kenema hospital 01/01/2013 to 01/01/2017.

FOIA Case No. 50125 - Any/all reports or evaluation of the effectiveness/reliability of Ebola tests/assays by members of the Viral Hemorrhagic Fever Consortium (i.e. Autoimmune Technologies, Broad Institute of MIT and Harvard University, Corgenix, The University of Texas Medical Branch at Galveston (UTMB), The Scripps Research Institute, the University of California at San Diego, the Irrua Specialist Teaching Hospital Lassa Fever Program, and the Kenema Government Hospital).

FOIA Case No. 50136 - The Institutional Review Board approvals related to Ebola sample collection or data collect from 01/01/2014 with either Robert F. Garry or John Schieffelin as Principal Investigators

This is a final response to **FOIA Case No. 50124**, your request for Any/all agreements (such as contracts, Memorandum of Understanding, Terms of Reference, Material Transfer Agreements etc) of NIAID with the Kenema hospital from 01/01/2013 to 01/01/2017. Any/all agreements (such as contracts, Memorandum of Understanding, Terms of Reference, Material Transfer Agreements etc.) of Tulane University with the Kenema hospital 01/01/2013 to 01/01/2017.

Staff in the following NIAID offices and divisions: Office of Global Relations; Division of Clinical Research; Division of Microbiology and Infectious Diseases; Division of Intramural Research; Division of Extramural Activities and the Technology Transfer and Intellectual Property Office searched their files and no records responsive to your request were located. While we believe that an adequate search of appropriate files was conducted for the records you requested, you have the right to appeal this determination that no records exist which would be responsive to your request. Should you wish to do so, your appeal must be sent within ninety (90) days of the date of this letter, following the procedures outlined in Subpart F of the HHS FOIA Regulations (https://www.federalregister.gov/documents/2016/10/28/2016-25684/freedom-of-information-regulations) to:

Deputy Agency Chief FOIA Officer
U.S. Department of Health and Human Services
Office of the Assistant Secretary for Public Affairs
Room 729H
200 Independence Avenue, S.W.
Washington, DC 20201
FOIARequest@hhs.gov
FAX: 202-690-8320

Clearly mark both the envelope and your letter "Freedom of Information Act Appeal."

If you are not satisfied with the processing and handling of this request, you may contact the NIAID FOIA Public Liaison and/or the Office of Government Information Services (OGIS):

NIAID FOIA Public Liaison
Margaret Moore
5601 Fishers Lane
Suite 6G51
Bethesda, MD 20892
301-451-5109 (phone)
301-480-0904 (fax)
Mm52s@nih.gov (email)

OGIS
National Archives and Records Admin.
8601 Adelphi Rd – OGIS
College Park, MD 20740-6001
202-741-5770 (phone)
1-877-684-6448 (toll-free)
202-741-5769 (fax)
ogis@nara.gov (email)

In certain circumstances provisions of the FOIA and Department of Health and Human Services FOIA Regulations allow us to recover part of the cost of responding to your request. Because no unusual circumstances apply to the processing of your request, there are no charges associated with our response.

Sincerely,

Personal Info

Robin L. Schofield
FOIA Coordinator
National Institute of Allergy and Infectious Diseases

OMB Approval 2700-0042

AWARD/CONTRACT	1. THIS CONTRACT IS A RATED ORDER UNDER DPAS (15 CFR 350) ▶	RATING N/A	PAGE 1 OF PAGES 48
2. CONTRACT (Proc. Inst. Ident.) NO. HHSN272200900049C	3. EFFECTIVE DATE September 30, 2009	4. REQUISITION/PURCHASE REQUEST/PROJECT NO. 1272934	

5. ISSUED BY CODE
National Institutes of Health
Office of Acquisitions (OA), NIAID, NIH, DHHS
Room 3214
6700-B Rockledge Dr., MSC 7612
Bethesda, Maryland 20892-7612

6. ADMINISTERED BY (If other than Item 6) CODE
AIT-RCB

7. NAME AND ADDRESS OF CONTRACTOR (No. street, county, state and ZIP Code)
Tulane University
1430 Tulane Avenue
New Orleans, LA 70112

8. DELIVERY
☐ FOB ORIGIN ☒ OTHER (See below)
FOB Destination

9. DISCOUNT FOR PROMPT PAYMENT
N/A

10. SUBMIT INVOICES ADDRESS SHOWN IN: ITEM Art. G.3.

CODE FACILITY CODE N/A

11. SHIP TO/MARK FOR CODE
Article F.2.

12. PAYMENT WILL BE MADE BY CODE N/A
See Article G.3.

13. AUTHORITY FOR USING OTHER FULL AND OPEN COMPETITION:
☐ 10 U.S.C. 2304(c)( ) ☐ 41 U.S.C. 253(c)( )

14. ACCOUNTING AND APPROPRIATION DATA

15A. ITEM NO.	15B. SUPPLIES/SERVICES	15C. QUANTITY	15D. UNIT	15E. UNIT PRICE	15F. AMOUNT
	Title: Roles of protective or pathogenic B cell epitopes in human Lassa Fever Period: September 30, 2009 through September 29, 2014 Contract Type: Cost-Reimbursement - Completion	FY 09 FY 10 FY 11 FY 12 FY 13	$3,000,693 $2,669,676 $3,004,048 $3,204,719 $3,345,791		

15G. TOTAL AMOUNT OF CONTRACT ▶ $15,224,927

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CONTRACTING OFFICER WILL COMPLETE ITEM 17 OR 18 AS APPLICABLE

17. ☒ CONTRACTOR'S NEGOTIATED AGREEMENT *(Contractor is required to sign this document and return __3__ copies to issuing office.)* Contractor agrees to furnish and deliver all items or perform all the services set forth or otherwise identified above and on any continuation sheets for the consideration stated herein. The rights and obligations of the parties to this contract shall be subject to and governed by the following documents: (a) this award/contract, (b) the solicitation, if any, and (c) such provisions, representations, certifications, and specifications, as are attached or incorporated by reference herein. *(Attachments are listed herein.)*

18. ☐ AWARD *(Contractor is not required to sign this document.)* Your offer on Solicitation Number _____ including the additions or changes made by you which additions or changes are set forth in full above, is hereby accepted as to the items listed above and on any continuation sheets. This award consummates the contract which consists of the following documents: (a) the Government's solicitation and your offer, and (b) this award/contract. No further contractual document is necessary.

19A. NAME AND TITLE OF SIGNER *(Type or print)*
KATHLEEN M. KOZAR
DIRECTOR, RESEARCH ADMINISTRATION

20A. NAME OF CONTRACTING OFFICER
Donald E. Collie
Contracting Officer, AIT-RCB, OA, NIAID, NIH, DHHS

19B. NAME OF CONTRACTOR
Personal Info
(Signature of person authorized to sign)

19C. DATE SIGNED 9/28/09

20B. UNITED STATES OF AMERICA
BY _____
(Signature of Contracting Officer)

20C. DATE SIGNED 28 Sep 09

CONTRACT TABLE OF CONTENTS

SECTION B - SUPPLIES OR SERVICES AND PRICES/COSTS

ARTICLE B.1. BRIEF DESCRIPTION OF SUPPLIES OR SERVICES

The goal of this project is to identify novel B cell epitopes on Lassa vius (LASV) proteins and elucidate mechanisms of antibody-mediated protection or pathogenesis in humans with Lassa fever (LF).

ARTICLE B.2. ESTIMATED COST

a. The estimated cost of this contract is $15,254,919 .

b. Total funds currently available for payment and allotted to this contract are $3,000,694. For further provisions on funding see the LIMITATION OF FUNDS clause referenced in Part II, ARTICLE I.2. Authorized Substitutions of Clauses.

c. It is estimated that the amount currently allotted will cover performance of the contract through 09/29/2010.

d. The Contracting Officer may allot additional funds to the contract without the concurrence of the Contractor.

ARTICLE B.3. PROVISIONS APPLICABLE TO DIRECT COSTS

a. **Items Unallowable Unless Otherwise Provided**

Notwithstanding the clauses, ALLOWABLE COST AND PAYMENT, incorporated in this contract, unless authorized in writing by the Contracting Officer, the costs of the following items or activities shall be unallowable as direct costs:

1. Acquisition, by purchase or lease, of any interest in real property;

2. Special rearrangement or alteration of facilities;

3. Purchase or lease of **any** item of general purpose office furniture or office equipment regardless of dollar value. (General purpose equipment is defined as any items of personal property which are usable for purposes other than research, such as office equipment and furnishings, pocket calculators, etc.);

4. Travel to attend general scientific meetings;

5. Foreign travel - See subparagraph b. below;

6. Consultant costs;

7. Subcontracts;

8. Patient care costs;

9. Accountable Government property (defined as both real and personal property with an acquisition cost of $1,000 or more and a life expectancy of more than two years) and "sensitive items" (defined

and listed in the Contractor's Guide for Control of Government Property), regardless of acquisition value.

10. Light Refreshment and Meal Expenditures

Requests to use contract funds to provide light refreshments and/or meals to either federal or nonfederal employees must be submitted to the Contracting Officer's Technical Representative (COTR), with a copy to the Contracting Officer, at least six (6) weeks in advance of the event. The request shall contain the following information: (a) name, date, and location of the event at which the light refreshments and/or meals will be provided; (b) a brief description of the purpose of the event; (c) a cost breakdown of the estimated light refreshment and/or meal costs; (d) the number of nonfederal and federal attendees receiving light refreshments and/or meals; and (e) if the event will be held somewhere other than a government facility, provide an explanation of why the event is not being held at a government facility.

Refer to NIH Manual Chapter 1160-1, Entertainment, for more information on NIH's policy on the use of appropriated funds for light refreshments and meals.

b. **Travel Costs**

1. Domestic Travel

a. Total expenditures for domestic travel (transportation, lodging, subsistence, and incidental expenses) incurred in direct performance of this contract shall not exceed $293,806 without the prior written approval of the Contracting Officer.

b. The Contractor shall invoice and be reimbursed for all travel costs in accordance with OMB Circular A-21 - "Cost Principles for Educational Institutions."

2. Foreign Travel

Requests for foreign travel must be submitted at least six weeks in advance and shall contain the following: (a) meeting(s) and place(s) to be visited, with costs and dates; (b) name(s) and title(s) of Contractor personnel to travel and their functions in the contract project; (c) contract purposes to be served by the travel; (d) how travel of Contractor personnel will benefit and contribute to accomplishing the contract project, or will otherwise justify the expenditure of NIH contract funds; (e) how such advantages justify the costs for travel and absence from the project of more than one person if such are suggested; and (f) what additional functions may be performed by the travelers to accomplish other purposes of the contract and thus further benefit the project.

ARTICLE B.4. ADVANCE UNDERSTANDINGS

Other provisions of this contract notwithstanding, approval of the following items within the limits set forth is hereby granted without further authorization from the Contracting Officer.

a. **Subcontracts**

Subcontract - Subcontractor Info

To negotiate a cost reimbursement type subcontract with Subcontractor Info for B cell epitote research for an amount not to exceed Subcontractor Info for the period 9/30/2009 - 9/29/2014. Award of the subcontract

shall not proceed without the prior written consent of the Contracting Officer upon review of the supporting documentation required by FAR Clause 52.244-2, Subcontracts. After receiving written consent of the subcontract by the Contracting Officer, a copy of the signed, executed subcontract shall be provided to the Contracting Officer.

Subcontract - Subcontracto

To negotiate a cost reimbursement type subcontract with Subcontrac tor for an amount not to exceed Subcontractor for the period 9/30/2009-9/29/2014 . Award of the subcontract shall not proceed without the prior written consent of the Contracting Officer upon review of the supporting documentation required by FAR Clause 52.244-2, Subcontracts. After receiving written consent of the subcontract by the Contracting Officer, a copy of the signed, executed subcontract shall be provided to the Contracting Officer.

Subcontract - Subcontractor Info

To negotiate a cost reimbursement type subcontract with Subcontractor Info for an amount not to exceed Subcontract 00 for the period 9/30/2009-9/29/2014 . Award of the subcontract shall not proceed without the prior written consent of the Contracting Officer upon review of the supporting documentation required by FAR Clause 52.244-2, Subcontracts. After receiving written consent of the subcontract by the Contracting Officer, a copy of the signed, executed subcontract shall be provided to the Contracting Officer.

Subcontract - Subcontractor Info

To negotiate a cost reimbursement type subcontract with Subcontractor Info for an amount not to exceed Subcontractor for the period 9/30/2009-9/29/2014 . Award of the subcontract shall not proceed without the prior written consent of the Contracting Officer upon review of the supporting documentation required by FAR Clause 52.244-2, Subcontracts. After receiving written consent of the subcontract by the Contracting Officer, a copy of the signed, executed subcontract shall be provided to the Contracting Officer.

Subcontract - Subcontractor Info

To negotiate a cost reimbursement type subcontract with Subcontractor Info for an amount not to exceed Subcontractor Info for the period 9/30/2009-9/29/2014 . Award of the subcontract shall not proceed without the prior written consent of the Contracting Officer upon review of the supporting documentation required by FAR Clause 52.244-2, Subcontracts. After receiving written consent of the subcontract by the Contracting Officer, a copy of the signed, executed subcontract shall be provided to the Contracting Officer.

Subcontract - Subcontractor Info

To negotiate a cost reimbursement type subcontract with Subcontractor Info Inc for an amount not to exceed Subcontractor Info for the period 9/30/2009-9/29/2014 . Award of the subcontract shall not proceed without the prior written consent of the Contracting Officer upon review of the supporting documentation required by FAR Clause 52.244-2, Subcontracts. After receiving written consent of the subcontract by the Contracting Officer, a copy of the signed, executed subcontract shall be provided to the Contracting Officer.

Subcontract - Subcontrac

To negotiate a cost reimbursement type subcontract with Subcontra for an amount not to exceed Subcontractor for the period 9/30/2009-9/29/2014 . Award of the subcontract shall not proceed without the prior written consent

of the Contracting Officer upon review of the supporting documentation required by FAR Clause 52.244-2, Subcontracts. After receiving written consent of the subcontract by the Contracting Officer, a copy of the signed, executed subcontract shall be provided to the Contracting Officer

b. **Invoices - Cost and Personnel Reporting, and Variances from the Negotiated Budget**

1. The Contractor agrees to provide a detailed breakdown on invoices of the following cost categories:

   a. Direct Labor - List individuals by name, title/position, hourly/annual rate, level of effort, and amount claimed.

   b. Fringe Benefits - Cite rate and amount

   c. Overhead - Cite rate and amount

   d. Materials & Supplies - Include detailed breakdown when total amount is over $1,000.

   e. Travel - Identify travelers, dates, destination, purpose of trip, and amount. Cite COA, if appropriate. List separately, domestic travel, general scientific meeting travel, and foreign travel.

   f. Consultant Fees - Identify individuals and amounts.

   g. Subcontracts - Attach subcontractor invoice(s).

   h. Equipment - Cite authorization and amount.

   i. G&A - Cite rate and amount.

   j. Total Cost

   k. Fixed Fee

   l. Total CPFF

   Monthly invoices must include the cumulative total expenses to date, adjusted (as applicable) to show any amounts suspended by the Government.

2. The Contractor agrees to immediately notify the Contracting Officer in writing if there is an anticipated overrun (any amount) or unexpended balance (greater than 10 percent) of the amount allotted to the contract, and the reasons for the variance. Also refer to the requirements of the Limitation of Funds and Limitation of Cost Clauses in the contract.

c. **Confidential Treatment of Sensitive Information**

The Contractor shall guarantee strict confidentiality of the information/data that it is provided by the Government during the performance of the contract. The Government has determined that the information/data that the Contractor will be provided during the performance of the contract is of a sensitive nature.

Disclosure of the information/data, in whole or in part, by the Contractor can only be made after the Contractor receives prior written approval from the Contracting Officer. Whenever the Contractor is uncertain with regard to the proper handling of information/data under the contract, the Contractor shall obtain a written determination from the Contracting Officer.

d. **Special Copyright Provisions**

1. In accordance with FAR Clause 52.227-14, Rights in Data General, the Contractor shall seek written permission from the Contracting Officer before establishing a copyright for any software and associated data generated under this contract. Additionally, the Government shall be provided a paid-up, world-wide, irrevocable, nonexclusive license to all rights under any copyright obtained.

e. **Contract Number Designation**

On all correspondence submitted under this contract, the Contractor agrees to clearly identify the two contract numbers that appear on the face page of the contract as follows:

Contract No. HHSN272200900049C

f. **Multi-year Contracts**

The cancellation date for each program year is the anniversary date of the contract award; the Contracting Officer will notify the Contractor 30 days in advance of the Governments intent to fund the next program year. Additionally, in accordance with FAR clause 52.217-2, Cancellation Under Multi-year Contracts, the Government has established the cancellation ceilings at 0% for Years 1, 2, 3, 4, and 5.

g. **Advance Copies of Press Releases**

Specific elements of cost, which normally require prior written approval of the Contracting Officer before incurrence of the cost (e.g., foreign travel, consultant fees, subcontracts) will be included inthis Article if the Contracting Officer has granted his/her approval prior to contract award.

The contractor agrees to accurately and factually represent the work conducted under this contract in all press releases. In accordance with NIH Manual Chapter 1754, misrepresenting contract results or releasing information that is injurious to the integrity of NIH may be construed as improper conduct. The complete text of NIH Manual Chapter 1754 can be found at: http://www1.od.nih.gov/oma/manualchapters/management/1754/

Press releases shall be considered to include the public release of information to any medium, excluding peer-reviewed scientific publications. The contractor shall ensure that the Contracting Officer's Technical Representative (COTR) has received an advance copy of any press release related to this contract not less than four (4) working days prior to the issuance of the press release.

SECTION C - DESCRIPTION/SPECIFICATIONS/WORK STATEMENT

ARTICLE C.1. STATEMENT OF WORK

a. Independently and not as an agent of the Government, the Contractor shall furnish all the necessary services, qualified personnel, material, equipment, and facilities, not otherwise provided by the Government as needed to perform the Statement of Work, dated September 30, 2009, set forth in SECTION J-List of Attachments, attached hereto and made a part of this contract.

b. Privacy Act System of Records Number 09-25-0087 is applicable to this contract and shall be used in any design, development, or operation work to be performed under the resultant contract. Disposition of records shall be in accordance with SECTION C of the contract, and by direction of the Contracting Officer's Technical Representative (COTR).

ARTICLE C.2. REPORTING REQUIREMENTS

All reports required herein shall be submitted in electronic format. In addition, one (1) hardcopy of each report shall be submitted to the Contracting Officer, unless otherwise specified.

a. **Technical Reports**

In addition to those reports required by the other terms of this contract, the Contractor shall prepare and submit the following reports in the manner stated below and in accordance with the DELIVERIES Article in SECTION F and ATTACHMENT 14 of this contract:

[Note: Beginning May 25, 2008, the Contractor shall include, in any technical progress report submitted, the applicable PubMed Central (PMC) or NIH Manuscript Submission reference number when citing publications that arise from its NIH funded research.]

1. **Quarterly Progress Report**

   a. This report shall include a description of the activities during the reporting period and the activities planned for the ensuing reporting period. The first reporting period consists of the first full three months of performance including any fractional part of the initial month. Thereafter, the reporting period shall consist of three full calendar months.

   b. A monthly report will not be submitted for the final month of the quarter.

   c. The first report shall be due 31 December 2009. Thereafter, reports shall be due on or before the 15th Calendar day following each reporting period.

2. **Semi-Annual Progress Report**

   a. This report shall include a description of the activities during the reporting period and the activities planned for the ensuing reporting period. The initial report will be submitted for the first full six months of the contract performance including any fractional part of the initial month. Thereafter, the reporting period shall consist of six full calendar months.

   b. Quarterly reports will not be submitted the month the semi-annual report is due.

   c. The first report shall be due 1 April 2010. Thereafter, reports shall be due on or before the 15th Calendar day following each reporting period.

3. **Annual Progress Report**

   This report shall include a summation of the results of the entire contract work for the period covered. An annual report will not be required for the period when the Final Report is due. A Quarterly Report shall not be submitted when an Annual Report is due.

   The first report shall cover the period 30 September 2009 through 29 September 2010 of this contract and shall be due within 30 days after the Anniversary Date of the Contract. Thereafter, reports shall be due on or before the 30th Calendar day following the reporting period.

4. **Annual Technical Progress Report for Clinical Research Study Populations**

   The Contractor shall submit information about the inclusion of women and members of minority groups and their subpopulations for each study being performed under this contract. The Contractor shall submit this information in the format indicated in the attachment entitled, "Inclusion Enrollment Report," which is set forth in SECTION J of this contract. The Contractor also shall use this format, modified to indicate that it is a final report, for reporting purposes in the final report.

   The Contractor shall submit the report in accordance with the DELIVERIES Article in SECTION F of this contract.

   In addition, the NIH Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research, Amended, October, 2001 applies. If this contract is for Phase III clinical trials, see II.B of these guidelines. The Guidelines may be found at the following website:

   http://grants.nih.gov/grants/funding/women_min/guidelines_amended_10_2001.htm

   Include a description of the plans to conduct analyses, as appropriate, by sex/gender and/or racial/ethnic groups in the clinical trial protocol as approved by the IRB, and provide a description of the progress in the conduct of these analyses, as appropriate, in the annual progress report and the final report. If the analysis reveals no subset differences, a brief statement to that effect, indicating the subsets analyzed, will suffice. The Government strongly encourages inclusion of the results of subset analysis in all publication submissions. In the final report, the Contractor shall include all final analyses of the data on sex/gender and race/ethnicity.

5. **Final Report**
   This report is to include a summation of the work performed and results obtained for the entire contract period of performance. This report shall be in sufficient detail to describe comprehensively the results achieved. The Final Report shall be submitted in accordance with the DELIVERIES Article in SECTION F of this contract. An Annual report will not be required for the period when the Final Report is due.

The Contractor shall provide the Contracting Officer with 2 copies of the Final Report in **draft** form 60 Calendar days prior to the expiration date of this contract. The Contracting Officer's Technical Representative (COTR) will review the draft report and provide the Contracting Officer with comments within 20 Calendar days after receipt. The Final Report shall be corrected by the Contractor, if necessary, and the final version delivered as specified in the above paragraph.

6. **Summary of Salient Results**

   The Contractor shall submit, with the Final Report, a summary (not to exceed 200 words) of salient results achieved during the performance of the contract.

7. **Report on Select Agents or Toxins and/or Highly Pathogenic Agents**

For work involving the possession, use, or transfer of a *Select Agent or Toxin* and/or a *Highly Pathogenic Agent*, the following information shall also be included in each AnnualProgress Report:

1. Any changes in the use of the *Select Agent or Toxin* and/or a *Highly Pathogenic Agent,* that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by the IBC or equivalent body or institutional biosafety official.
2. If work with a new or additional *Select Agent or Toxin* and/or a Highly Pathogenic Agent will be conducted in the upcoming reporting period, provide:
    a. A list of each new or additional *Select Agent or Toxin* and/or a *Highly Pathogenic Agent* that will be studied;
    b. A description of the work that will be done with each new or additional *Select Agent or Toxin* and/or a *Highly Pathogenic Agent*;
    c. The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or institutional biosafety official. It must be noted if the work is being done in a new location.

If the IBC or equivalent body or institutional biosafety official has determined, for example, by conducting a risk assessment, that the work that has been performed or is planned to be performed under this contract may be conducted at a biocontainment safety level that is lower than BSL3, a statement to that affect shall be included in each Annual Progress Report.

If no work involving a Select Agent or Toxin and/or a Highly Pathogenic Agent has been performed or is planned to be performed under this contract, a statement to that affect shall be included in each Annual Progress Report.

1. **Source Code and Object Code**

Unless otherwise specified herein, the Contractor shall deliver to the Government, upon the expiration date of the contract, all source code and object code developed, modified, and/or enhanced under this contract.

2. **Information Security Reporting Requirements**

The Contractor shall submit the following reports as required by the INFORMATION SECURITY Article in SECTION H of this contract. Note: Each report listed below includes a reference to the appropriate subparagraph of this article.

a. **Roster of Employees Requiring Suitability Investigations**

The Contractor shall submit a roster, by name, position, e-mail address, phone number and responsibility, of all staff (including subcontractor staff) working under the contract who will develop, have the ability to access, or host and/or maintain a Federal information system(s). The roster shall be submitted to the Contracting Officer's Technical Representative (COTR), with a copy to the Contracting Officer, within 14 calendar days of the effective date of the contract. Any revisions to the roster as a result of staffing changes shall be submitted within 15 calendar days of the change. (Reference subparagraph c.(2) of the INFORMATION SECURITY Article in SECTION H of this contract.)

b. **Information Security Training Report**

The Contractor shall maintain a listing by name and title of each employee (including subcontractors) working under this contract that has completed the NIH required information security training. Any additional security training completed by Contractor/ Subcontractor staff shall be included on this listing. [The listing of completed training shall be included in the first technical progress report. (See Article C.2.a. Technical Progress Reports.) Any revisions to this listing as a result of staffing changes shall be submitted with next required technical progress report.] (Reference subparagraph d. of the INFORMATION SECURITY Article in SECTION H of this contract.)

c. **Reporting of New and Departing Employees**

The Contractor shall notify the Contracting Officer's Technical Representative (COTR) and Contracting Officer within five business days of staffing changes for positions that require suitability determinations as follows (Reference subparagraph f. of the INFORMATION SECURITY Article in SECTION H of this contract.):

(1) **New Employees:** Provide the name, position title, e-mail address, and phone number of the new employee. Provide the name, position title and suitability level held by the former incumbent. If the employee is filling a new position, provide a description of the position and the Government will determine the appropriate security level.

(2) **Departing Employees:** 1) Provide the name, position title, and security clearance level held by or pending for the individual; and 2) Perform and document the actions identified in the "Employee Separation Checklist", attached in Section J, ATTACHMENTS of this contract, when a Contractor/Subcontractor employee terminates work under this contract. All documentation shall be made available to the COTR and/or Contracting Officer upon request

d. **Contractor - Employee Non-Disclosure Agreement(s)**

The contractor shall complete and submit a signed and witnessed "Commitment to Protect Non-Public Information - Contractor Agreement" form for each contractor and subcontractor employee who may have access to non-public Department information under this contract. This form is located at: http://ocio.nih.gov/security/Nondisclosure.pdf. (Reference subparagraph g.of the INFORMATION SECURITY Article in SECTION H of this contract.)

e. **Self Assessment & Information Security Plan Reporting**

(1) **NIST SP 800-53 Self-Assessment** (Reference subparagraph h. of the INFORMATION SECURITY Article in SECTION H of this contract.)

The contractor shall annually update and resubmit its Self-Assessment required by NIST SP 800-53, Recommended Security Controls for Federal Information Systems to the Contracting Officer's Technical Representative (COTR), with a copy to the Contracting Officer [For option contracts: no later than the completion date of the period of performance/ for all other contracts: indicate due date as determined by the COTR/ Contracting Officer]. (http://csrc.nist.gov/publications - under Special Publications).

The Contractor's annual update to its Self-Assessment Questionnaire shall include similar information for any subcontractor that performs under the SOW to (1) develop a Federal information system(s) at the Contractor's/Subcontractor's facility, or (2) host and/or maintain a Federal information system(s) at the Contractor's/Subcontractor's facility.

(2) **Information System Security Plan** (Reference subparagraph i. of the INFORMATION SECURITY Article in SECTION H of this contract.)

The Contractor's draft ISSP submitted with its proposal shall be finalized in coordination with the COTR [insert date/no later than 90 calendar days after contract award].

Following approval of its draft ISSP, the Contractor shall update and resubmit its ISSP to the COTR every three years or when a major modification has been made to its internal system. The Contractor shall use the current ISSP template in Appendix A of NIST SP 800-18, Guide to Developing Security Plans for Federal Information Systems. (http://csrc.nist.gov/publications/nistpubs/800-18-Rev1/sp800-18-Rev1-final.pdf).

The Contractor shall include similar information for any subcontractor performing under the SOW with the Contractor whenever the submission of an ISSP is required.

3. **Epitope Data**
Immune epitope information shall be submitted to the IEDB (www.ImmuneEpitope.org) within six (6) months of validation, or as determined by the Project Officer, in consulation with the Contracting Officer and Contractor. This information includes:

a) epitope sequence and any post-translational modifications important for epitope recognition or antigen processing;

b) pathogen species and strain from which the epitope was identified;

c) antigen from which the epitope was derived;

d) epitope indentification methods;

e) validation methods (e.g.,test recognition by antibodies, and role in protective immunity) and summary of results; and

f) extensive annotation may also be required, as determined in conslultation with Project Officer. This annotation may include: three-deminsional structure of the antigen or epitope; epitope location on the whole antigen; pathogen replication state in which the epitope is expressed; and disease state in which the expressed or recognized by the host.

Prediction Tools

Contract generated software shall be made available, within 6 months of validation, through publicly accessibly web and databases sites, with first preference given to the IEDB (www.immuneEpitope.org). If the IEDB does not choose to host the tool, it shall be made accessible through other public websites or repositories identified by the Contractor in consultation with the Project Officer. Contract generated software includes, but is not limited to:

a) epitope prediction algorithms;

b) artificial neural networks;

c) epitope visualization tools; and

d) mathematical models.

To the extent possible, the Contractor shall provide software under a license certified by the Open Source Initiative (http://www.opensource.org/licenses/) to guarantee the right to read, redistribute, modify and freely use the software. In addition, these computational tools shall have a user interface that allows the scientific community to use the tools in their individual research projects.

The Contractor shall be solely responsible for the timely acquisition of all appropriate proprietary rights, including intellectual property rights, and all materials needed to perform the project. Before, during, and subsequent to the award, the U.S. Government is not required to obtain for the Contractor any proprietary rights, including intellectual property rights, or any materials needed by the U.S.

Government all inventions made in the performance of the project, as specified at FAR 52.227-11 (Bayh-Dole Act).

Intellectual Property

The Contractor shall be solely responsible for the timely acquisition of all appropriate proprietary rights, including intellectual property rights, and all materials needed to perform the project. Before, during, and subsequent to the award, the U.S. Government is not required to obtain for the Contractor any proprietary rights, including intellectual property rights, or any materials needed by the Contractor to perform the project. The Contractor is required to report to the U.S. Government all inventions made in the performance of the project, as specified at FAR 52.227-11 (Bayh-Dole Act).

Other Reports and Deliverables may be required. This may include special reports required by regulation or policy and deliverables such as data, vaccine, etc.

ARTICLE C.3. INVENTION REPORTING REQUIREMENT

All reports and documentation required by FAR Clause 52.227-11, Patent Rights-Ownership by the Contractor including, but not limited to, the invention disclosure report, the confirmatory license, and the Government support certification, shall be directed to the Extramural Inventions and Technology Resources Branch, OPERA, NIH, 6705 Rockledge Drive, Room 2207, MSC 7987, Bethesda, Maryland 20892-7987 (Telephone: 301-435-1986). In addition, one copy of an annual utilization report, and a copy of the final invention statement, shall be submitted to the Contracting Officer. The final invention statement (see FAR 27.303(b)(2)(ii)) shall be submitted to the Contracting Officer on the expiration date of the contract.

The annual utilization report shall be submitted in accordance with the DELIVERIES Article in SECTION F of this contract. Thereafter, reports shall be due on or before the 30 Calendar day following the reporting period.] The final invention statement (see FAR 27.303(b)(2)(ii)) shall be submitted on the expiration date of the contract. All reports shall be sent to the following address:

Contracting Officer
National Institutes of Health
National Institute of Allergy and Infectious Diseases
Office of Acquisitions
6700-B Rockledge Drive
Room 3109
Bethesda, Maryland 20892- 7612

If no invention is disclosed or no activity has occurred on a previously disclosed invention during the applicable reporting period, a negative report shall be submitted to the Contracting Officer at the address listed above.

To assist contractors in complying with invention reporting requirements of the clause, the NIH has developed "Interagency Edison," an electronic invention reporting system. Use of Interagency Edison is encouraged as it streamlines the reporting process and greatly reduces paperwork. Access to the system is through a secure interactive Web site to ensure that all information submitted is protected. Interagency Edison and information relating to the capabilities of the system can be obtained from the Web (http://www.iedison.gov), or by contacting the Extramural Inventions and Technology Resources Branch, OPERA, NIH.

SECTION D - PACKAGING, MARKING AND SHIPPING

All deliverables required under this contract shall be packaged, marked and shipped in accordance with Government specifications. At a minimum, all deliverables shall be marked with the contract number and Contractor name. The Contractor shall guarantee that all required materials shall be delivered in immediate usable and acceptable condition.

SECTION E - INSPECTION AND ACCEPTANCE

a. The Contracting Officer or the duly authorized representative will perform inspection and acceptance of materials and services to be provided.

b. For the purpose of this SECTION, Contracting Officer Technical Representative (COTR is the authorized representative of the Contracting Officer.

c. Inspection and acceptance will be performed at:
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland

Acceptance may be presumed unless otherwise indicated in writing by the Contracting Officer or the duly authorized representative within 30 days of receipt.

d. This contract incorporates the following clause by reference, with the same force and effect as if it were given in full text. Upon request, the Contracting Officer will make its full text available.

*FAR Clause **52.246-9, Inspection of Research and Development (Short Form)** (April 1984).*

SECTION F - DELIVERIES OR PERFORMANCE

ARTICLE F.1. DELIVERIES

Satisfactory performance of the final contract shall be deemed to occur upon performance of the work described in the Statement of Work Article in SECTION C of this contract and upon delivery and acceptance by the Contracting Officer, or the duly authorized representative, of the following items in accordance with the stated delivery schedule:

a. The items specified in ATTACHMENT 14, Deliverables, as described in the REPORTING REQUIREMENTS Article in SECTION C of this contract will be required to be delivered F.o.b. Destination as set forth in FAR 52.247-35, F.o.b. DESTINATION, WITHIN CONSIGNEES PREMISES (APRIL 1984), and in accordance with and by the dates specified in ATTACHMENT 14.

ARTICLE F.2. CLAUSES INCORPORATED BY REFERENCE, FAR 52.252-2 (FEBRUARY 1998)

This contract incorporates the following clause(s) by reference, with the same force and effect as if it were given in full text. Upon request, the Contracting Officer will make its full text available. Also, the full text of a clause may be accessed electronically at this address: http://www.acquisition.gov/comp/far/index.html

FEDERAL ACQUISITION REGULATION (48 CFR CHAPTER 1) CLAUSE:

52.242-15, Stop Work Order (August 1989) with **Alternate I** (April 1984).

SECTION G - CONTRACT ADMINISTRATION DATA

ARTICLE G.1. CONTRACTING OFFICER'S TECHNICAL REPRESENTATIVE (COTR)

The following Contracting Officer's Technical Representative(s) (COTR(s)) will represent the Government for the purpose of this contract:

Program Officer, Basic Immunology Branch
Division of Allergy, Immunology and Transplantation
NIAID, NIH
Room 3004
6610 Rockledge Drive
Bethesda, MD 20892-6601
Phone: 301-451-3103
Fax: 301-480-2381
E-mail: fergusonst@niaid.nih.gov

The COTR is responsible for: (1) monitoring the Contractor's technical progress, including the surveillance and assessment of performance and recommending to the Contracting Officer changes in requirements; (2) interpreting the statement of work and any other technical performance requirements; (3) performing technical evaluation as required; (4) performing technical inspections and acceptances required by this contract; and (5) assisting in the resolution of technical problems encountered during performance.

The Contracting Officer is the only person with authority to act as agent of the Government under this contract. Only the Contracting Officer has authority to: (1) direct or negotiate any changes in the statement of work; (2) modify or extend the period of performance; (3) change the delivery schedule; (4) authorize reimbursement to the Contractor for any costs incurred during the performance of this contract; or (5) otherwise change any terms and conditions of this contract.

The Government may unilaterally change its COTR designation.

ARTICLE G.2. KEY PERSONNEL, HHSAR 352.270-5 (January 2006)

The key personnel specified in this contract are considered to be essential to work performance. At least 30 days prior to diverting any of the specified individuals to other programs or contracts (or as soon as possible, if an individual must be replaced, for example, as a result of leaving the employ of the Contractor), the Contractor shall notify the Contracting Officer and shall submit comprehensive justification for the diversion or replacement request (including proposed substitutions for key personnel) to permit evaluation by the Government of the impact on performance under this contract. The Contractor shall not divert or otherwise replace any key personnel without the written consent of the Contracting Officer. The Government may modify the contract to add or delete key personnel at the request of the Contractor or Government.

(End of Clause)

The following individual(s) is/are considered to be essential to the work being performed hereunder:

Name	Title
James E. Robinson, MD	Principle Investigator

ARTICLE G.3. INVOICE SUBMISSION/CONTRACT FINANCING REQUEST AND CONTRACT FINANCIAL REPORT

a. Invoice/Financing Request Instructions and Contract Financial Reporting for NIH Cost-Reimbursement Type Contracts NIH(RC)-4 are attached and made part of this contract. The Contractor shall follow the attached instructions and submission procedures specified below to meet the requirements of a "proper invoice" pursuant to FAR Subpart 32.9, Prompt Payment.

1. Payment requests shall be submitted to the offices identified below. **Do not submit supporting documentation (e.g., receipts, time sheets, vendor invoices, etc.) with your payment request unless specified elsewhere in the contract or requested by the Contracting Officer.**

   a. The original invoice shall be submitted to the following **designated billing office**:

      National Institutes of Health
      Office of Financial Management
      Commercial Accounts
      2115 East Jefferson Street, Room 4B-432, MSC 8500
      Bethesda, MD 20892-8500

   b. One copy of the invoice shall be submitted to the following **approving official**:

      Contracting Officer
      Office of Acquisitions
      National Institute of Allergy and Infectious Diseases
      6700B Rockledge Drive Room 3214
      BETHESDA, MD 20892

      NIAIDOAinvoices@niaid.nih.gov

      The Contractor shall submit an electronic copy of the payment request to the approving official instead of a paper copy. The payment request shall be transmitted as an attachment via e-mail to the address listed above in one of the following formats: MSWord, MS Excel, or Adobe Portable Document Format (PDF). Only one payment request shall be submitted per e-mail and the subject line of the e-mail shall include the Contractor's name, contract number, and unique invoice number. ***[Note: The original payment request must still be submitted in hard copy and mailed to the designated billing office to meet the requirements of a "proper invoice."]***

2. In addition to the requirements specified in FAR 32.905 for a proper invoice, the Contractor shall include the following information on the face page of all payment requests:

   a. Name of the Office of Acquisitions. The Office of Acquisitions for this contract is NIAID .

   b. Central Point of Distribution. For the purpose of this contract, the Central Point of Distribution is NIAIDOAInvoices .

   c. Federal Taxpayer Identification Number (TIN). If the Contractor does not have a valid TIN, it shall identify the Vendor Identification Number (VIN) on the payment request. The VIN is the number that appears after the Contractor's name on the face page of the contract. *[Note: A VIN is assigned to new contracts awarded on or after June 4, 2007, and any existing contract modified to include the VIN number.]* If the Contractor has neither a TIN, DUNS, or VIN, contact the Contracting Officer.

   d. DUNS or DUNS+4 Number. The DUNS number must identify the Contractor's name and address exactly as stated in the contract and as registered in the Central Contractor Registration (CCR) database. If the Contractor does not have a valid DUNS number, it shall identify the Vendor Identification Number (VIN) on the payment request. The VIN is the number that appears after the Contractor's name on the face page of the contract. *[Note: A VIN is assigned to new contracts awarded on or after June 4, 2007, and any existing contract modified to include*

the VIN number.] If the Contractor has neither a TIN, DUNS, or VIN, contact the Contracting Officer.

e. Invoice Matching Option. This contract requires a two-way match.

f. Unique Invoice Number. Each payment request must be identified by a unique invoice number, which can only be used one time regardless of the number of contracts or orders held by an organization.

b. Inquiries regarding payment of invoices shall be directed to the designated billing office, (301) 496-6452.

ARTICLE G.4. INDIRECT COST RATES

In accordance with Federal Acquisition Regulation (FAR) (48 CFR Chapter 1) Clause 52.216-7 (d)(2), Allowable Cost and Payment incorporated by reference in this contract in PART II, SECTION I, the cognizant Contracting Officer representative responsible for negotiating provisional and/or final indirect cost rates is identified as follows:

Director, Division of Financial Advisory Services
Office of Acquisition Management and Policy
National Institutes of Health
6100 Building, Room 6B05
6100 EXECUTIVE BLVD MSC-7540
BETHESDA MD 20892-7540

These rates are hereby incorporated without further action of the Contracting Officer.

ARTICLE G.5. GOVERNMENT PROPERTY

a. In addition to the requirements of the clause, GOVERNMENT PROPERTY, incorporated in SECTION I of this contract, the Contractor shall comply with the provisions of HHS Publication, "Contractor's Guide for Control of Government Property," which is incorporated into this contract by reference. This document can be accessed at:

http://www.hhs.gov/oamp/policies/contractors_guide_for_control_of_gov_property.pdf.
Among other issues, this publication provides a summary of the Contractor's responsibilities regarding purchasing authorizations and inventory and reporting requirements under the contract. A copy of this publication is available upon request to the Contracts Property Administrator.

Requests for information regarding property under this contract should be directed to the following office:

Division of Personal Property Services, NIH
6011 Building, Suite 637
6011 EXECUTIVE BLVD MSC 7670
BETHESDA MD 20892-7670
(301) 496-6466

b. Notwithstanding the provisions outlined in the HHS Publication, "Contractor's Guide for Control of Government Property," which is incorporated in this contract in paragraph a. above, the Contractor shall use the form entitled, "Report of Government Owned, Contractor Held Property" for submitting summary reports required under this contract, as directed by the Contracting Officer or his/her designee. This form is included as an attachment in SECTION J of this contract.

c. **Contractor-Acquired Government Property - Schedule I-A**

Pursuant to the clause, GOVERNMENT PROPERTY, incorporated in this contract, the Contractor is hereby authorized to acquire the property listed in the attached Schedule I-A for use in direct performance of the contract. Schedule I-A is included as an attachment in SECTION J of this contract.

ARTICLE G.6. POST AWARD EVALUATION OF CONTRACTOR PERFORMANCE

a. Contractor Performance Evaluations

Interim and final evaluations of Contractor performance will be prepared on this contract in accordance with FAR Subpart 42.15. The final performance evaluation will be prepared at the time of completion of work. In addition to the final evaluation, interim evaluation(s) shall be submitted by the COTR and CO, at least once during the performance of the contract.

Interim and final evaluations will be provided to the Contractor as soon as practicable after completion of the evaluation. The Contractor will be permitted thirty days to review the document and to submit additional information or a rebutting statement. If agreement cannot be reached between the parties, the matter will be referred to an individual one level above the Contracting Officer, whose decision will be final.

Copies of the evaluations, Contractor responses, and review comments, if any, will be retained as part of the contract file, and may be used to support future award decisions.

b. Electronic Access to Contractor Performance Evaluations

Contractors that have Internet capability may access evaluations through a secure Web site for review and comment by completing the registration form that can be obtained at the following address:

http://oamp.od.nih.gov/OD/CPS/cps.asp

The registration process requires the Contractor to identify an individual that will serve as a primary contact and who will be authorized access to the evaluation for review and comment. In addition, the Contractor will be required to identify an alternate contact who will be responsible for notifying the cognizant contracting official in the event the primary contact is unavailable to process the evaluation within the required 30-day time frame.

SECTION H - SPECIAL CONTRACT REQUIREMENTS

ARTICLE H.1. PROTECTION OF HUMAN SUBJECTS, HHSAR 352.270-8(b) (January 2006)

(a) The Contractor agrees that the rights and welfare of human subjects involved in research under this contract shall be protected in accordance with 45 CFR Part 46 and with the Contractor's current Assurance of Compliance on file with the Office for Human Research Protections (OHRP), Office of Public Health and Science (OPHS). The Contractor further agrees to provide certification at least annually that the Institutional Review Board has reviewed and approved the procedures, which involve human subjects in accordance with 45 CFR Part 46 and the Assurance of Compliance.

(b) The Contractor shall bear full responsibility for the performance of all work and services involving the use of human subjects under this contract and shall ensure that work is conducted in a proper manner and as safely as is feasible. The parties hereto agree that the Contractor retains the right to control and direct the performance of all work under this contract. Nothing in this contract shall be deemed to constitute the Contractor or any subcontractor, agent or employee of the Contractor, or any other person, organization, institution, or group of any kind whatsoever, as the agent or employee of the Government. The Contractor agrees that it has entered into this contract and will discharge its obligations, duties, and undertakings and the work pursuant thereto, whether requiring professional judgement or otherwise, as an independent contractor without imputing liability on the part of the Government for the acts of the Contractor or its employees.

(c) If at any time during the performance of this contract, the Contracting Officer determines, in consultation with the OHRP, OPHS, ASH, that the Contractor is not in compliance with any of the requirements and/or standards stated in paragraphs (a) and (b) above, the Contracting Officer may immediately suspend, in whole or in part, work and further payments under this contract until the Contractor corrects the noncompliance. Notice of the suspension may be communicated by telephone and confirmed in writing. If the Contractor fails to complete corrective action within the period of time designated in the Contracting Officer's written notice of suspension, the Contracting Officer may, in consultation with OHRP, OPHS, ASH, terminate this contract in a whole or in part, and the Contractor's name may be removed form the list of those contractors with approved Health and Human Services Human Subject Assurances.

(End of clause)

ARTICLE H.2. RESTRICTION ON USE OF HUMAN SUBJECTS, HHSAR 352.270-14 (January 2006)

Pursuant to 45 CFR part 46, Protection of Human Research Subjects, the Contractor shall not expend funds under this award for research involving human subjects or engage in any human subjects research activity prior to the receipt by the Contracting Officer of a certification that the research has been reviewed and approved by the Institutional Review Board (IRB) designated under the Contractor's Federal-wide assurance of compliance. This restriction applies to all collaborating sites, whether domestic or foreign, and subcontractors. The Contractor must ensure compliance by collaborators and subcontractors.
(End of clause)

Prisoners shall not be enrolled in any HHS research activities until all requirements of HHS Regulations at 45 CFR PART 46, Subpart C Have been met. If a Research Subject becomes a prisoner during the period of this contract, 45 CFR PART 46, Subpart C will apply to research involving that individual.

ARTICLE H.3. REQUIRED EDUCATION IN THE PROTECTION OF HUMAN RESEARCH PARTICIPANTS

NIH policy requires education on the protection of human subject participants for all investigators receiving NIH contract awards for research involving human subjects. For a complete description of the NIH Policy announcement on required education in the protection of human subject participants, the Contractor should access the NIH Guide for Grants and Contracts Announcement dated June 5, 2000 at the following website:

http://grants.nih.gov/grants/guide/notice-files/NOT-OD-00-039.html.

The information below is a summary of the NIH Policy Announcement:

The Contractor shall maintain the following information: (1) a list of the names and titles of the principal investigator and any other individuals working under the contract who are responsible for the design and/or conduct of the research; (2) the title of the education program(s) in the protection of human subjects that has been completed for each named personnel and; (3) a one sentence description of the educational program(s) listed in (2) above. This requirement extends to investigators and all individuals responsible for the design and/or conduct of the research who are working as subcontractors or consultants under the contract.

Prior to any substitution of the Principal Investigator or any other individuals responsible for the design and/or conduct of the research under the contract, the Contractor shall provide the following written information to the Contracting Officer: the title of the education program and a one sentence description of the program that has been completed by the replacement.

ARTICLE H.4. HUMAN MATERIALS

The acquisition and supply of all human specimen material (including fetal material) used under this contract shall be obtained by the Contractor in full compliance with applicable State and Local laws and the provisions of the Uniform Anatomical Gift Act in the United States, and no undue inducements, monetary or otherwise, will be offered to any person to influence their donation of human material.

ARTICLE H.5. HUMAN MATERIALS (ASSURANCE OF OHRP COMPLIANCE)

The acquisition and supply of all human specimen material (including fetal material) used under this contract shall be obtained by the Contractor in full compliance with applicable State and Local laws and the provisions of the Uniform Anatomical Gift Act in the United States, and no undue inducements, monetary or otherwise, will be offered to any person to influence their donation of human material.

The Contractor shall provide written documentation that all human materials obtained as a result of research involving human subjects conducted under this contract, by collaborating sites, or by subcontractors identified under this contract, were obtained with prior approval by the Office for Human Research Protections (OHRP) of an Assurance to comply with the requirements of 45 CFR 46 to protect human research subjects. This restriction applies to all collaborating sites without OHRP-approved Assurances, whether domestic or foreign, and compliance must be ensured by the Contractor.

Provision by the Contractor to the Contracting Officer of a properly completed "Protection of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption", Form OMB No. 0990-0263(formerly Optional Form 310), certifying IRB review and approval of the protocol from which the human materials were obtained constitutes the written documentation required. The human subject certification can be met by submission of a self designated form, provided that it contains the information required by the "Protection of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption", Form OMB No. 0990-0263(formerly Optional Form 310).

ARTICLE H.6. SALARY RATE LIMITATION LEGISLATION PROVISIONS

a. The Contractor shall not use NIH Fiscal Year funds to pay the direct salary of an individual through this contract at a rate in excess of Executive Level I. Direct salary is exclusive of fringe benefits, overhead and general and administrative expenses (also referred to as "indirect costs" or "facilities and administrative (F&A) costs"). Direct salary has the same meaning as the term "institutional base salary." An individual's direct salary (or institutional base salary) is the annual compensation that the Contractor pays for an individual's appointment whether that individual's time is spent on research, teaching, patient care or other activities. Direct salary (or institutional base salary) excludes any income that an individual may be permitted to earn outside of duties to the Contractor. The annual salary rate limitation also applies to individuals proposed under subcontracts. It does not apply to fees paid to consultants. If this is a multiple year contract, it may be subject to unilateral modifications by the Government if an individual's salary rate used to establish contract funding exceeds any salary rate limitation subsequently established in future HHS appropriation acts.

b. Payment of direct salaries is limited to the Executive Level I rate which was in effect on the date(s) the expense was incurred. See the following Web site for Executive Schedule rates of pay: http://www.opm.gov/oca/. *(For current year rates, click on Salaries and Wages / Executive Schedule / Rates of Pay for the Executive Schedule. For prior year rates, click on Salaries and Wages / cursor to bottom of page and select year / Executive Schedule / Rates of Pay for the Executive Schedule. Rates are effective January 1 of each calendar year unless otherwise noted.)*

ARTICLE H.7. NEEDLE EXCHANGE

The Contractor shall not use contract funds to carry out any program of distributing sterile needles or syringes for the hypodermic injection of any illegal drug.

ARTICLE H.8. PRESS RELEASES

The Contractor shall clearly state, when issuing statements, press releases, requests for proposals, bid solicitations and other documents describing projects or programs funded in whole or in part with Federal money: (1) the percentage of the total costs of the program or project which will be financed with Federal money; (2) the dollar amount of Federal funds for the project or program; and (3) the percentage and dollar amount of the total costs of the project or program that will be financed by nongovernmental sources.

ARTICLE H.9. RESTRICTION ON ABORTIONS

The Contractor shall not use contract funds for any abortion.

ARTICLE H.10. CONTINUED BAN ON FUNDING OF HUMAN EMBRYO RESEARCH

The Contractor shall not use contract funds for (1) the creation of a human embryo or embryos for research purposes; or (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFR 46.204(b) and Section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)). The term "human embryo or embryos" includes any organism, not protected as a human subject under 45 CFR 46 as of the date of the enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells.

Additionally, in accordance with a March 4, 1997 Presidential Memorandum, Federal funds may not be used for cloning of human beings.

ARTICLE H.11. DISSEMINATION OF FALSE OR DELIBERATELY MISLEADING SCIENTIFIC INFORMATION

The Contractor shall not use contract funds to disseminate scientific information that is deliberately false or misleading.

ARTICLE H.12. RESTRICTION ON EMPLOYMENT OF UNAUTHORIZED ALIEN WORKERS

The Contractor shall not use contract funds to employ workers described in section 274A(h)(3) of the Immigration and Nationality Act, which reads as follows:

> "(3) Definition of unauthorized alien. - As used in this section, the term 'unauthorized alien' means, with respect to the employment of an alien at a particular time, that the alien is not at that time either (A) an alien lawfully admitted for permanent residence, or (B) authorized to be so employed by this Act or by the Attorney General."

ARTICLE H.13. PRIVACY ACT, HHSAR 352.270-11 (January 2006)

This contract requires the Contractor to perform one or more of the following: (a) Design; (b) develop; or (c) operate a Federal agency system of records to accomplish an agency function in accordance with the Privacy Act of 1974

(Act) (5 U.S.C. 552a(m)(1)) and applicable agency regulations. The term "system of records" means a group of any records under the control of any agency from which information is retrieved by the name of the individual or by some identifying number, symbol, or other identifying particular assigned to the individual.

Violations of the Act by the Contractor and/or its employees may result in the imposition of criminal penalties (5 U.S.C. 552a(i)). The Contractor shall ensure that each of its employees knows the prescribed rules of conduct and that each employee is aware that he/she is subject to criminal penalties for violation of the Act to the same extent as HHS employees. These provisions also apply to all subcontracts awarded under this contract which require the design, development or operation of the designated system(s) of records (5 U.S.C. 552a(m)(1)).

The contract work statement: (a) Identifies the system(s) of records and the design, development, or operation work to be performed by the Contractor; and (b) specifies the disposition to be made of such records upon completion of contract performance.

(End of clause)

45 CFR Part 5b contains additional information which includes the rules of conduct and other Privacy Act requirements and can be found at: http://www.access.gpo.gov/nara/cfr/waisidx_06/45cfr5b_06.html.

The Privacy Act System of Records applicable to this project is Number _____. This document is incorporated into this contract as an Attachment in SECTION J of this contract. This document is also available at: http://oma.od.nih.gov/ms/privacy/pa-files/read02systems.htm.

ARTICLE H.14. CARE OF LIVE VERTEBRATE ANIMALS, HHSAR 352.270-9(b) (January 2006)

(a) Before undertaking performance of any contract involving animal related activities, the Contractor shall register with the Secretary of Agriculture of the United States in accordance with 7 U.S.C. 2136 and 9 CFR 2.25 through 2.28. The Contractor shall furnish evidence of the registration to the Contracting Officer.

(b) The Contractor shall acquire vertebrate animals used in research from a dealer licensed by the Secretary of Agriculture under 7 U.S.C. 2133 and 9 CFR 2.1 through 2.11, or from a source that is exempt from licensing under those sections.

(c) The Contractor agrees that the care and use of any live vertebrate animals used or intended for use in the performance of this contract will conform with the PHS Policy on Humane Care of Use of Laboratory Animals, the current Animal Welfare Assurance, the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and the pertinent laws and regulations of the United States Department of Agriculture (*see* 7 U.S.C. 2131 *et seq.* and 9 CFR Subchapter A, Parts 1 - 4). In case of conflict between standards, the more stringent standard shall be used.

(d) If at any time during performance of this contract, the Contracting Officer determines, in consultation with the Office of Laboratory Animal Welfare (OLAW), National Institutes of Health (NIH), that the Contractor is not in compliance with any of the requirements and/or standards stated in paragraphs (a) through (c) above, the Contracting Officer may immediately suspend, in whole or in part, work and further payments under this contract until the Contractor corrects the noncompliance. Notice of the suspension may be communicated by telephone and confirmed in writing. If the Contractor fails to complete corrective action within the period of time designated in the Contracting Officer's written notice of suspension, the Contracting Officer may, in consultation with OLAW, NIH, terminate this contract in whole or in part, and the Contractor's name may be removed from the list of those contractors with approved PHS Animal Welfare Assurances.

Note: The Contractor may request registration of its facility and a current listing of licensed dealers from the Regional Office of the Animal and Plant Health Inspection Service (APHIS), USDA, for the region in which its research facility is located. The location of the appropriate APHIS Regional Office, as well as information concerning this program may be obtained by contacting the Animal Care Staff, USDA/APHIS, 4700 River Road, Riverdale, Maryland 20737.

(End of Clause)

ARTICLE H.15. ANIMAL WELFARE

All research involving live, vertebrate animals shall be conducted in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. This policy may be accessed at:

http://grants1.nih.gov/grants/olaw/references/phspol.htm.

ARTICLE H.16. INTRODUCTION OF RODENTS AND RODENT PRODUCTS

No rodent or rodent product shall be delivered into the NIH, ___ environment (NIH) directly, or through collaborative research or holding facilities under contract to ___ except by permit. Direct shipments to NIH from a Division of Veterinary Resources (DVR), Office of Research Services (ORS) approved source will be considered exempt. Non-exempt sources must be approved by permit issued through the DVR, ORS. The permit must be obtained by the Contractor prior to the shipment to NIH of the rodents and/or rodent products. The Contractor must be sure that this permit exists and is current before transferring rodents or rodent products into the NIH, ___ environment. Refusal or negligence to do so will be considered a material breach of contract and may be treated as any other such material breach. Applications for permits should be submitted by facsimile not less than 30 days prior (60 days in situations where quarantine is likely) to shipping date to: NIH Division of Veterinary Resources (DVR), Office of Research Services (ORS), Building 14G, Service Rd. South, Room 102, BETHESDA MD 20892-5210, (301)496-2527, FAX: (301) 402-0352.

ARTICLE H.17. PROTECTION OF PERSONNEL WHO WORK WITH NONHUMAN PRIMATES

All Contractor personnel who work with nonhuman primates or enter rooms or areas containing nonhuman primates shall comply with the procedures set forth in NIH Policy Manual 3044-2, entitled, "Protection of NIH Personnel Who Work with Nonhuman Primates," located at the following URL:

http://www1.od.nih.gov/oma/manualchapters/intramural/3044-2/

ARTICLE H.18. SUBCONTRACTING PROVISIONS

a. **Small Business Subcontracting Plan**

1. The Small Business Subcontracting Plan, dated 30 September 2009 is attached hereto and made a part of this contract.
2. The failure of any Contractor or subcontractor to comply in good faith with FAR Clause 52.219-8, entitled "Utilization of Small Business Concerns" incorporated in this contract and the attached Subcontracting Plan, will be a material breach of such contract or subcontract and subject to the remedies reserved to the Government under FAR Clause 52.219-16 entitled, "Liquidated Damages-Subcontracting Plan."

b. **Subcontracting Reports**

The Contractor shall submit the following Subcontracting reports electronically via the "electronic Subcontracting Reporting System (eSRS) at http://www.esrs.gov.

1. Individual Subcontract Reports (ISR)

   Regardless of the effective date of this contract, the Report shall be due on the following dates for the entire life of this contract:

   April 30th
   October 30th
   Expiration Date of Contract

2. Summary Subcontract Report (SSR)

   Regardless of the effective date of this contract, the Summary Subcontract Report shall be submitted annually on the following date for the entire life of this contract:

October 30th

For both the Individual and Summary Subcontract Reports, the Contracting Officer shall be included as a contact for notification purposes at the following e-mail address:

dcollie@niaid.nih.gov

ARTICLE H.19. INFORMATION SECURITY

The Statement of Work (SOW) requires the Contractor to (1) develop, (2) have the ability to access, or (3) host and/ or maintain a Federal information system(s). Pursuant to Federal and HHS Information Security Program Policies, the Contractor and any subcontractor performing under this contract shall comply with the following requirements:

Federal Information Security Management Act of 2002 (FISMA), Title III, E-Government Act of 2002, Pub. L. No. 107-347 (Dec. 17, 2002); http://csrc.nist.gov/drivers/documents/FISMA-final.pdf

a. Information Type

[X] Administrative, Management and Support Information

Scientific and Technical Research and Innovation

b. Security Categories and Levels

Confidentiality Level:	[X] Low [ ] Moderate [ ] High
Integrity Level:	[X] Low [ ] Moderate [ ] High
Availability Level:	[X] Low [ ] Moderate [ ] High
Overall Level:	[X] **Low** [ ] **Moderate** [ ] **High**

c. Position Sensitivity Designations

1. The following position sensitivity designations and associated clearance and investigation requirements apply under this contract.

   [ ] **Level 6: Public Trust - High Risk (Requires Suitability Determination with a BI)**. Contractor employees assigned to a Level 6 position are subject to a Background Investigation (BI)

   [ ] **Level 5: Public Trust - Moderate Risk (Requires Suitability Determination with NACIC, MBI or LBI).** Contractor employees assigned to a Level 5 position with no previous investigation and approval shall undergo a National Agency Check and Inquiry Investigation plus a Credit Check (NACIC), a Minimum Background Investigation (MBI), or a Limited Background Investigation (LBI).

   [X] **Level 1: Non Sensitive (Requires Suitability Determination with an NACI).** Contractor employees assigned to a Level 1 position are subject to a National Agency Check and Inquiry Investigation (NACI).

2. The Contractor shall submit a roster, by name, position, e-mail address, phone number and responsibility, of all staff (including subcontractor staff) working under the contract who will develop, have the ability to access, or host and/or maintain a Federal information system(s). The roster shall be submitted to the Contracting Officer's Technical Representative (COTR), with a copy to the Contracting Officer, within 14 calendar days of the effective date of the contract. Any revisions to the roster as a result of staffing changes shall be submitted within 15 calendar days of the change. The Contracting Officer shall notify

the Contractor of the appropriate level of suitability investigations to be performed. An electronic template, "Roster of Employees Requiring Suitability Investigations," is available for Contractor use at: http://ais.nci.nih.gov/forms/Suitability-roster.xls.

Upon receipt of the Government's notification of applicable Suitability Investigations required, the Contractor shall complete and submit the required forms within 30 days of the notification. Additional submission instructions can be found at the "NCI Information Technology Security Policies, Background Investigation Process" website: http://ais.nci.nih.gov.

Contractor/subcontractor employees who have met investigative requirements within the past five years may only require an updated or upgraded investigation.

3. Contractor/Subcontractor employees shall comply with the HHS criteria for the assigned position sensitivity designations prior to performing any work under this contract. The following exceptions apply:

   Levels 5 and 1: Contractor/Subcontractor employees may begin work under the contract after the Contractor has submitted the name, position and responsibility of the employee to the COTR, as described in paragraph c. (2) above.

   Level 6: In special circumstances the COTR may request a waiver of the pre-appointment investigation. If the waiver is granted, the COTR will provide written authorization for the Contractor/Subcontractor employee to work under the contract.

d. Information Security Training

The Contractor shall ensure that each Contractor/Subcontractor employee has completed the NIH Computer Security Awareness Training course at: http://irtsectraining.nih.gov/ prior to performing any contract work, and thereafter completing the NIH-specified fiscal year refresher course during the period of performance of the contract.

The Contractor shall maintain a listing by name and title of each Contractor/Subcontractor employee working under this contract that has completed the NIH required training. Any additional security training completed by Contractor/Subcontractor staff shall be included on this listing. [The listing of completed training shall be included in the first technical progress report. (See Article C.2. Reporting Requirements.) Any revisions to this listing as a result of staffing changes shall be submitted with next required technical progress report.]

Contractor/Subcontractor staff shall complete the following additional training prior to performing any work under this contract:

e. Rules of Behavior

The Contractor/Subcontractor employees shall comply with the NIH Information Technology General Rules of Behavior at: http://irm.cit.nih.gov/security/nihitrob.html.

f. Personnel Security Responsibilities

Contractor Notification of New and Departing Employees Requiring Background Investigations

1. The Contractor shall notify the Contracting Officer, the Contracting Officer's Technical Representative (COTR), and the Security Investigation Reviewer **within five working days** before a new employee assumes a position that requires a suitability determination or when an employee with a security clearance stops working under the contract. The Government will initiate a background investigation on new employees requiring security clearances and will stop pending background investigations for employees that no longer work under the contract.
2. New employees: Provide the name, position title, e-mail address, and phone number of the new employee. Provide the name, position title and suitability level held by the former incumbent. If the employee is filling

a new position, provide a description of the position and the Government will determine the appropriate security level.

3. Departing employees:

   - Provide the name, position title, and security clearance level held by or pending for the individual.
   - Perform and document the actions identified in the "Employee Separation Checklist", attached in Section J, ATTACHMENTS of this contract, when a Contractor/Subcontractor employee terminates work under this contract. All documentation shall be made available to the COTR and/or Contracting Officer upon request.

g. Commitment to Protect Non-Public Departmental Information Systems and Data

1. Contractor Agreement

   The Contractor and its subcontractors performing under this SOW shall not release, publish, or disclose non-public Departmental information to unauthorized personnel, and shall protect such information in accordance with provisions of the following laws and any other pertinent laws and regulations governing the confidentiality of such information:

   -18 U.S.C. 641 (Criminal Code: Public Money, Property or Records)
   -18 U.S.C. 1905 (Criminal Code: Disclosure of Confidential Information)
   -Public Law 96-511 (Paperwork Reduction Act)

2. Contractor-Employee Non-Disclosure Agreements

   Each Contractor/Subcontractor employee who may have access to non-public Department information under this contract shall complete the Commitment to Protect Non-Public Information - Contractor Agreement. A copy of each signed and witnessed Non-Disclosure agreement shall be submitted to the Contracting Officer's Technical Representative (COTR) prior to performing any work under the contract.

h. NIST SP 800-53 Self-Assessment

The contractor shall annually update and re-submit its Self-Assessment required by NIST SP 800-53, *Recommended Security Controls for Federal Information Systems.* (http://csrc.nist.gov/publications - under Special Publications).

Subcontracts: The Contractor's annual update to its Self-Assessment Questionnaire shall include similar information for any subcontractor that performs under the SOW to (1) develop a Federal information system(s) at the Contractor's/Subcontractor's facility, or (2) host and/or maintain a Federal information system(s) at the Contractor's/Subcontractor's facility.

The annual update shall be submitted to the Contracting Officer's Technical Representative (COTR), with a copy to the Contracting Officer [For option contracts: no later than the completion date of the period of performance/ for all other contracts: indicate due date as determined by the COTR/Contracting Officer].

i. Information System Security Plan

The Contractor's draft ISSP submitted with its proposal shall be finalized in coordination with the Contracting Officer's Technical Representative (COTR) no later than 90 calendar days after contract award.

Following approval of its draft ISSP, the Contractor shall update and resubmit its ISSP to the COTR every three years or when a major modification has been made to its internal system. The Contractor shall use the current ISSP template in Appendix A of NIST SP 800-18, Guide to Developing Security Plans for Federal Information Systems. (http://csrc.nist.gov/publications/nistpubs/800-18-Rev1/sp800-18-Rev1-final.pdf). The details contained in the Contractor's ISSP shall be commensurate with the size and complexity of the requirements of the SOW based on the System Categorization determined above in subparagraph (b) Security Categories and Levels of this Article.

Subcontracts: The Contractor shall include similar information for any subcontractor performing under the SOW with the Contractor whenever the submission of an ISSP is required.

j. Common Security Configurations

The contractor shall ensure that any information technology acquired under this contract incorporates the applicable common security configuration established by the National Institute of Standards and Technology (NIST) at http://checklists.nist.gov.

ARTICLE H.20. ELECTRONIC AND INFORMATION TECHNOLOGY ACCESSIBILITY (January 2008)

Pursuant to Section 508 of the Rehabilitation Act of 1973 (29 U.S.C. 794d), as amended by the Workforce Investment Act of 1998, all electronic and information technology (EIT) products and services developed, acquired, maintained, and/or used under this contract/order must comply with the "Electronic and Information Technology Accessibility Provisions" set forth by the Architectural and Transportation Barriers Compliance Board (also referred to as the "Access Board") in 36 CFR part 1194. Information about Section 508 provisions is available at http://www.section508.gov/. The complete text of Section 508 Final provisions can be accessed at http://www.access-board.gov/sec508/provisions.htm.

The Section 508 standards applicable to this contract/order are identified in the Statement of Work. The contractor must provide a written Section 508 conformance certification due at the end of each order/contract exceeding $100,000 when the order/contract duration is one year or less. If it is determined by the Government that EIT products and services provided by the Contractor do not conform to the described accessibility in the Product Assessment Template, remediation of the products and/or services to the level of conformance specified in the vendor's Product Assessment Template will be the responsibility of the Contractor at its own expense.

In the event of a modification(s) to the contract/order, which adds new EIT products and services or revised the type of, or specifications for, products and services the Contractor is to provide, including EIT deliverables such as electronic documents and reports, the Contracting Officer may require that the contractor submit a completed HHS Section 508 Product Assessment Template to assist the Government in determining that the EIT products and services support Section 508 accessibility requirements. Instructions for documenting accessibility via the HHS Section 508 Product Assessment Template may be found at http://508.hhs.gov.

[(End of HHSAR 352.270-19(b)]

Prior to the Contracting Officer exercising an option for a subsequent performance period/additional quantity or adding increment funding for a subsequent performance period under this contract, as applicable, the Contractor must provide a Section 508 Annual Report to the Contracting Officer and Contracting Officer's Technical Representative (COTR). Unless otherwise directed by the Contracting Officer in writing, the Contractor shall provide the cited report in accordance with the following schedule. Instructions for completing the report are available at: http://508.hhs.gov/ under the heading Vendor Information and Documents. The Contractor's failure to submit a timely and properly completed report may jeopardize the Contracting Officer's exercising an option or adding incremental funding, as applicable.

Schedule for Contractor Submission of Section 508 Annual Report:

[End of HHSAR 352.270-19(c)]

ARTICLE H.21. ACCESS TO NATIONAL INSTITUTES OF HEALTH (NIH) ELECTRONIC MAIL

All Contractor staff that have access to and use of NIH electronic mail (e-mail) must identify themselves as contractors on all outgoing e-mail messages, including those that are sent in reply or are forwarded to another user. To best comply with this requirement, the Contractor staff shall set up an e-mail signature ("AutoSignature") or an electronic

business card ("V-card") on each Contractor employee's computer system and/or Personal Digital Assistant (PDA) that will automatically display "Contractor" in the signature area of all e-mails sent.

ARTICLE H.22. CONFIDENTIALITY OF INFORMATION

The following information is covered by **HHSAR 352.224-70, Confidentiality of Information** (January 2006):

ARTICLE H.23. PUBLICATION AND PUBLICITY

In addition to the requirements set forth in HHSAR Clause **352.270-6, Publications and Publicity** incorporated by reference in SECTION I of this contract, the Contractor shall acknowledge the support of the National Institutes of Health whenever publicizing the work under this contract in any media by including an acknowledgment substantially as follows:

> "This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN272200900049C"

ARTICLE H.24. REPORTING MATTERS INVOLVING FRAUD, WASTE AND ABUSE

Anyone who becomes aware of the existence or apparent existence of fraud, waste and abuse in NIH funded programs is encouraged to report such matters to the HHS Inspector General's Office in writing or on the Inspector General's Hotline. The toll free number is **1-800-HHS-TIPS (1-800-447-8477).** All telephone calls will be handled confidentially. The e-mail address is **Htips@os.dhhs.gov** and the mailing address is:

> Office of Inspector General
> Department of Health and Human Services
> TIPS HOTLINE
> P.O. Box 23489
> Washington, D.C. 20026

ARTICLE H.25. YEAR 2000 COMPLIANCE

In accordance with FAR 39.106, Information Technology acquired under this contract must be Year 2000 compliant as set forth in the following clause(s):

ARTICLE H.26. OBTAINING AND DISSEMINATING BIOMEDICAL RESEARCH RESOURCES

Unique research resources arising from NIH-funded research are to be shared with the scientific research community. NIH provides guidance, entitled, "Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources: Final Notice," (Federal Register Notice, December 23, 1999 [64 FR 72090]), concerning the appropriate terms for disseminating and acquiring these research resources. This guidance, found at : http://www.ott.nih.gov/policy/rt_guide_final.aspx is intended to help contractors ensure that the conditions they impose and accept on the transfer of research tools will facilitate further biomedical research, consistent with the requirements of the Bayh-Dole Act and NIH funding policy.

Note: For the purposes of this Article, the terms, "research tools", "research materials", and "research resources" are used interchangeably and have the same meaning.

ARTICLE H.27. SHARING RESEARCH DATA

The data sharing plan submitted by the Contractor is acceptable. The Contractor agrees to adhere to its plan and shall request prior approval of the Contracting Officer for any changes in its plan.

The NIH endorses the sharing of final research data to serve health. this contract is expected to generate research data that must be shared with the public and other researchers. NIH's data sharing policy may be found at the following Web site:

http://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-032.html

NIH recognizes that data sharing may be complicated or limited, in some cases, by institutional policies, local IRB rules, as well as local, state and Federal laws and regulations, including the Privacy Rule (see HHS-published documentation on the Privacy Rule at http://www.hhs.gov/ocr/). The rights and privacy of people who participate in NIH-funded research must be protected at all times; thus, data intended for broader use should be free of identifiers that would permit linkages to individual research participants and variables that could lead to deductive disclosure of the identity of individual subjects.

ARTICLE H.28. POSSESSION USE AND TRANSFER OF SELECT BIOLOGICAL AGENTS OR TOXINS

The contractor shall not conduct work involving select agents or toxins under this contract until it and any associated subcontractor(s) comply with the following:

For prime or subcontract awards to ***domestic institutions*** that possess, use, and/or transfer Select Agents under this contract, the institution must comply with the provisions of 42 CFR part 73, 7 CFR part 331, and/or 9 CFR part 121 (http://www.aphis.usda.gov/programs/ag_selectagent/FinalRule3-18-05.pdf) as required, before using NIH funds for work involving a *Select Agent or Toxin*. **No NIH funds can be used for research involving a *Select Agent or Toxin* at a domestic institution without a valid registration certificate.**

For prime or subcontract awards to ***foreign institutions*** that possess, use, and/or transfer a *Select Agent or Toxin*, before using NIH funds for any work directly involving a *Select Agent or Toxin*, the foreign institution must provide information satisfactory to the NIAID that safety, security, and training standards equivalent to those described in 42 CFR part 73, 7 CFR part 331, and/or 9 CFR part 121 are in place and will be administered on behalf of all *Select Agent or Toxin* work supported by these funds. The process for making this determination includes inspection of the foreign laboratory facility by an NIAID representative. During this inspection, the foreign institution must provide the following information: concise summaries of safety, security, and training plans; names of individuals at the foreign institution who will have access to the Select Agents and procedures for ensuring that only approved and appropriate individuals, in accordance with institution procedures, will have access to the Select Agents under the contract; and copies of or links to any applicable laws, regulations, policies, and procedures applicable to that institution for the safe and secure possession, use, and/or transfer of select agents. **No NIH funds can be used for work involving a *Select Agent or Toxin* at a foreign institution without written approval from the Contracting Officer.**

Listings of HHS select agents and toxins, and overlap select agents or toxins as well as information about the registration process for domestic institutions, are available on the Select Agent Program Web site at http://www.cdc.gov/od/sap/ and http://www.cdc.gov/od/sap/docs/salist.pdf.

Listings of USDA select agents and toxins as well as information about the registration process for domestic institutions are available on the APHIS/USDA website at: http://www.aphis.usda.gov/programs/ag_selectagent/index.html and:
http://www.aphis.usda.gov/programs/ag_selectagent/ag_bioterr_forms.html

For foreign institutions, see the NIAID Select Agent Award information:
(http://www.niaid.nih.gov/ncn/clinical/default_biodefense.htm).

ARTICLE H.29. HOTEL AND MOTEL FIRE SAFETY ACT OF 1990 (P.L. 101-391)

Pursuant to Public Law 101-391, no Federal funds may be used to sponsor or fund in whole or in part a meeting, convention, conference or training seminar that is conducted in, or that otherwise uses the rooms, facilities, or services of a place of public accommodation that do not meet the requirements of the fire prevention and control

guidelines as described in the Public Law. This restriction applies to public accommodations both foreign and domestic.

Public accommodations that meet the requirements can be accessed at: http://www.usfa.fema.gov/hotel/index.htm.

ARTICLE H.30. PROHIBITION ON CONTRACTOR INVOLVEMENT WITH TERRORIST ACTIVITIES

The Contractor acknowledges that U.S. Executive Orders and Laws, including but not limited to E.O. 13224 and P.L. 107-56, prohibit transactions with, and the provision of resources and support to, individuals and organizations associated with terrorism. It is the legal responsibility of the Contractor to ensure compliance with these Executive Orders and Laws. This clause must be included in all subcontracts issued under this contract.

ARTICLE H.31. CONSTITUTION DAY

Each educational institution that receives Federal funds for a fiscal year shall hold an educational program on the United States Constitution on September 17 of such year for the students serviced by the educational institution in accordance with Public Law 108-447.

ARTICLE H.32. REGISTRATION FEES FOR NIH SPONSORED SCIENTIFIC, EDUCATIONAL, AND RESEARCH-RELATED CONFERENCES

In accordance with the NIH Reform Act of 2006, P.L. 109-482, the NIH may authorize a Contractor procured to assist in the development and implementation of a scientific, educational or research-related conference to collect and retain registration fees from Non-HHS Federal and Non-Federal participants to defray the costs of the contract.

Whenever possible, the Contracting Officer, prior to each conference, shall provide the Contractor with uniform assumptions of the government's estimate of the registration fee offset to include in the costs estimate for the conference. This offset should be deducted from the total cost of the conference.

Prior to each conference, the Contractor shall submit a completed "Contractor Pre-Conference Expense Offset Worksheet" (Attachment provided in SECTION J) to the Contracting Officer's Technical Representative (COTR) and Contracting Officer. After the conference is held, the Contractor shall submit a completed "Post-Conference Expense Offset Worksheet" (Attachment provided in SECTION J) to the COTR and Contracting Officer.

The Contractor shall collect and maintain current and accurate accounting of collected conference fees and conference expenses. The Contractor shall immediately notify the COTR and Contracting Officer, in writing, if it appears the total registration fees collected will exceed the estimated total cost of the conference. If the registration fees collected are in excess of the total actual conference expenditures, the contractor shall return the excess funds to the Contracting Officer to be deposited as miscellaneous receipts into the U.S. Treasury.

If the registration fees collected are in excess of the uniform assumptions provided by the Contracting Officer, the Contracting Officer, shall, as necessary, modify the contract price to reflect the decrease in conference costs. If the registration fees collected are less than the uniform assumptions provided by the Contracting Officer, the Contracting Officer shall, as necessary, modify the contract price to reflect the increase in conference costs.

Although Contractors may bill for allowable conference costs as they are incurred, they may not submit a final invoice for the total costs of the conference until the "Post-Conference Expense Offset Worksheet" has been approved by the COTR.

ARTICLE H.33. GUIDELINES FOR INCLUSION OF WOMEN, MINORITIES, AND PERSONS WITH DISABILITIES IN NIH-SUPPORTED CONFERENCES

Pursuant to the NIH Revitalization Act (P.L. 103-43, Section 206), which adds Section 402(b) to the Public Health Service Act, it is required that NIH, "in conducting and supporting programs for research, research training, recruitment, and other activities, provide for an increase in the number of women and individuals from disadvantaged backgrounds (including racial and ethnic minorities) in the fields of biomedical and behavioral research." In addition,

Section 504 of the Rehabilitation Act of 1973 and the Americans with Disabilities Act of 1990 require reasonable accommodations to be provided to individuals with disabilities.

It is NIH policy that organizers of scientific meetings should make a concerted effort to achieve appropriate representation of women, racial/ethnic minorities, and persons with disabilities, and other individuals who have been traditionally underrepresented in science, in all NIH sponsored and/or supported scientific meetings.

Therefore, it is the contractor's responsibility to ensure the inclusion of women, minorities, and persons with disabilities in all events when recruiting speakers and/or participants for meetings or conferences funded by this contract.

See the policy announcement for additional details and definitions at:
http://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-066.html

PART II - CONTRACT CLAUSES

ARTICLE I.1. General Clauses for a Cost-Reimbursement Research and Development Contract

This contract incorporates the following clauses by reference, with the same force and effect as if they were given in full text. Upon request, the Contracting Officer will make their full text available. Also, the full text of a clause may be accessed electronically at this address: http://www.arnet.gov/far/.

a. FEDERAL ACQUISITION REGULATION (FAR) (48 CFR CHAPTER 1) CLAUSES:

FAR CLAUSE NO.	DATE	TITLE
52.202-1	Jul 2004	Definitions (Over $100,000)
52.203-3	Apr 1984	Gratuities (Over $100,000)
52.203-5	Apr 1984	Covenant Against Contingent Fees (Over $100,000)
52.203-6	Sep 2006	Restrictions on Subcontractor Sales to the Government (Over $100,000)
52.203-7	Jul 1995	Anti-Kickback Procedures (Over $100,000)
52.203-8	Jan 1997	Cancellation, Rescission, and Recovery of Funds for Illegal or Improper Activity (Over $100,000)
52.203-10	Jan 1997	Price or Fee Adjustment for Illegal or Improper Activity (Over $100,000)
52.203-12	Sep 2007	Limitation on Payments to Influence Certain Federal Transactions (Over $100,000)
52.204-4	Aug 2000	Printed or Copied Double-Sided on Recycled Paper (Over $100,000)
52.204-7	Apr 2008	Central Contractor Registration
52.204-10	Sep 2007	Reporting Subcontract Awards ($500,000,000 or more)
52.209-6	Sep 2006	Protecting the Government's Interests When Subcontracting With Contractors Debarred, Suspended, or Proposed for Debarment (Over $30,000)
52.215-2	Mar 2009	Audit and Records - Negotiation [Note: Applies to ALL contracts funded in whole or in part with Recovery Act funds, regardless of dollar value, AND contracts over $100,000 funded exclusively with non-Recovery Act funds.]
52.215-8	Oct 1997	Order of Precedence - Uniform Contract Format
52.215-10	Oct 1997	Price Reduction for Defective Cost or Pricing Data (Over $650,000)
52.215-12	Oct 1997	Subcontractor Cost or Pricing Data (Over $650,000)
52.215-14	Oct 1997	Integrity of Unit Prices (Over $100,000)
52.215-15	Oct 2004	Pension Adjustments and Asset Reversions
52.215-18	Jul 2005	Reversion or Adjustment of Plans for Post-Retirement Benefits (PRB) other than Pensions
52.215-19	Oct 1997	Notification of Ownership Changes
52.215-21	Oct 1997	Requirements for Cost or Pricing Data or Information Other Than Cost or Pricing Data - Modifications
52.216-7	Dec 2002	Allowable Cost and Payment
52.216-8	Mar 1997	Fixed Fee
52.219-8	May 2004	Utilization of Small Business Concerns (Over $100,000)
52.219-9	Apr 2008	Small Business Subcontracting Plan (Over $550,000, $1,000,000 for Construction)

FAR CLAUSE NO.	DATE	TITLE
52.219-16	Jan 1999	Liquidated Damages - Subcontracting Plan (Over $550,000, $1,000,000 for Construction)
52.222-2	Jul 1990	Payment for Overtime Premium (Over $100,000) (Note: The dollar amount in paragraph (a) of this clause is $0 unless otherwise specified in the contract.)
52.222-3	Jun 2003	Convict Labor
52.222-21	Feb 1999	Prohibition of Segregated Facilities
52.222-26	Mar 2007	Equal Opportunity
52.222-35	Sep 2006	Equal Opportunity for Special Disabled Veterans, Veterans of the Vietnam Era, and Other Eligible Veterans (Over $100,000)
52.222-36	Jun 1998	Affirmative Action for Workers with Disabilities
52.222-37	Sep 2006	Employment Reports on Special Disabled Veterans, Veterans of the Vietnam Era, and Other Eligible Veterans (Over $100,000)
52.222-50	Feb 2009	Combating Trafficking in Persons
52.222-54	Jan 2009	Employment Eligibility Verification (Over $100,000)
52.223-6	May 2001	Drug-Free Workplace
52.223-14	Aug 2003	Toxic Chemical Release Reporting (Over $100,000)
52.225-1	Feb 2009	Buy American Act - Supplies
52.225-13	Jun 2008	Restrictions on Certain Foreign Purchases
52.227-1	Dec 2007	Authorization and Consent, Alternate I (Apr 1984)
52.227-2	Dec 2007	Notice and Assistance Regarding Patent and Copyright Infringement
52.227-11	Dec 2007	Patent Rights - Ownership by the Contractor (Note: In accordance with FAR 27.303(b)(2), paragraph (e) is modified to include the requirements in FAR 27.303(b)(2)(i) through (iv). The frequency of reporting in (i) is annual.
52.227-14	Dec 2007	Rights in Data - General
52.232-9	Apr 1984	Limitation on Withholding of Payments
52.232-17	Oct 2008	Interest (Over $100,000)
52.232-20	Apr 1984	Limitation of Cost
52.232-23	Jan 1986	Assignment of Claims
52.232-25	Oct 2008	Prompt Payment, Alternate I (Feb 2002)
52.232-33	Oct 2003	Payment by Electronic Funds Transfer--Central Contractor Registration
52.233-1	Jul 2002	Disputes
52.233-3	Aug 1996	Protest After Award, Alternate I (Jun 1985)
52.233-4	Oct 2004	Applicable Law for Breach of Contract Claim
52.242-1	Apr 1984	Notice of Intent to Disallow Costs
52.242-3	May 2001	Penalties for Unallowable Costs (Over $650,000)
52.242-4	Jan 1997	Certification of Final Indirect Costs
52.242-13	Jul 1995	Bankruptcy (Over $100,000)
52.243-2	Aug 1987	Changes - Cost Reimbursement, Alternate V (Apr 1984)
52.244-2	Jun 2007	Subcontracts, Alternate I (June 2007)
52.244-5	Dec 1996	Competition in Subcontracting (Over $100,000)

FAR CLAUSE NO.	DATE	TITLE
52.244-6	Mar 2009	Subcontracts for Commercial Items
52.245-1	Jun 2007	Government Property
52.245-9	Jun 2007	Use and Charges
52.246-23	Feb 1997	Limitation of Liability (Over $100,000)
52.249-6	May 2004	Termination (Cost-Reimbursement)
52.249-14	Apr 1984	Excusable Delays
52.253-1	Jan 1991	Computer Generated Forms

b. DEPARTMENT OF HEALTH AND HUMAN SERVICES ACQUISITION REGULATION (HHSAR) (48 CFR CHAPTER 3) CLAUSES:

HHSAR CLAUSE NO.	DATE	TITLE
352.202-1	Jan 2006	Definitions - with Alternate paragraph (h) (Jan 2006)
352.216-72	Jan 2006	Additional Cost Principles
352.228-7	Dec 1991	Insurance - Liability to Third Persons
352.232-9	Jan 2006	Withholding of Contract Payments
352.233-70	Jan 2006	Litigation and Claims
352.242-71	Apr 1984	Final Decisions on Audit Findings
352.270-5	Jan 2006	Key Personnel
352.270-6	Jan 2006	Publications and Publicity
352.270-10	Jan 2006	Anti-Lobbying (Over $100,000)

[End of GENERAL CLAUSES FOR A NEGOTIATED COST-REIMBURSEMENT RESEARCH AND DEVELOPMENT CONTRACT- Rev. 09/2009].

ARTICLE I.2. AUTHORIZED SUBSTITUTION OF CLAUSES

ARTICLE I.1. of this SECTION is hereby modified as follows:

a. FAR Clause **52.232-20, Limitation Of Cost** (April 1984), is deleted in its entirety and FAR Clause **52.232-22, Limitation Of Funds** (April 1984) is substituted therefor. **[NOTE: When this contract is fully funded, FAR Clause 52.232-22, LIMITATION OF FUNDS will no longer apply and FAR Clause 52.232-20, LIMITATION OF COST will become applicable.]**

b. FAR Clauses **52.249-6, Termination (Cost-Reimbursement)** (May 2004) and **52.249-14, Excusable Delays** (April 1984), are deleted in their entirety and FAR Clause **52.249-5, Termination for Convenience of the Government (Educational and Other Nonprofit Institutions)** (September 1996), is substituted therefore.

ARTICLE I.3. Additional Contract Clauses

This contract incorporates the following clauses by reference, with the same force and effect, as if they were given in full text. Upon request, the Contracting Officer will make their full text available.

a. FEDERAL ACQUISITION REGULATION (FAR) (48 CFR CHAPTER 1) CLAUSES

1. FAR Clause **52.203-13, Contractor Code of Business Ethics and Conduct** (December 2008).

2. FAR Clause **52.203-14, Display of Hotline Poster(s)** (December 2007).

   ".....(3) Any required posters may be obtained as follows:

Poster(s)	Obtain From"
HHS Contractor Code of Ethics and Business Conduct Poster	http://www.oig.hhs.gov/fraud/hotline/OIG_Hotline_Poster.pdf

3. FAR Clause **52.208-9, Contractor Use of Mandatory Sources of Supply or Services** (October 2008).

4. FAR Clause **52.216-15, Predetermined Indirect Cost Rates** (April 1998).

5. FAR Clause **52.217-2, Cancellation Under Multiyear Contracts** (October 1997).

6. FAR Clause **52.219-4, Notice of Price Evaluation Preference for HUBZone Small Business Concerns** (July 2005).

   "(c) Waiver of evaluation preference.....
   [ ] Offeror elects to waive the evaluation preference."

7. FAR Clause **52.219-25, Small Disadvantaged Business Participation Program--Disadvantaged Status and Reporting** (April 2008).

8. FAR Clause **52.224-1, Privacy Act Notification** (April 1984).

9. FAR Clause **52.224-2, Privacy Act** (April 1984).

10. FAR Clause **52.227-14, Rights in Data - General** (December 2007).

11. **Alternate II** (December 2007), FAR Clause **52.227-14, Rights in Data--General** (December 2007).

    Additional purposes for which the limited rights data may be used are:

12. **Alternate IV** (December 2007), FAR Clause **52.227-14, Rights in Data - General** (December 2007).

13. **Alternate V** (December 2007), FAR Clause **52.227-14, Rights in Data--General** (December 2007).

Specific data items that are not subject to paragraph (j) include:

14. FAR Clause **52.227-16, Additional Data Requirements** (June 1987).

15. FAR Clause **52.227-17, Rights in Data--Special Works** (December 2007).

16. FAR Clause **52.227-19, Commercial Computer Software License** (December 2007).

17. FAR Clause **52.229-8, Taxes-Foreign Cost-Reimbursement Contracts** (March 1990).

18. FAR Clause **52.230-2, Cost Accounting Standards** (October 2008).

19. FAR Clause **52.230-3, Disclosure and Consistency of Cost Accounting Practices** (October 2008).

20. FAR Clause **52.230-5, Cost Accounting Standards - Educational Institution** (October 2008).

21. FAR Clause **52.230-6, Administration of Cost Accounting Standards** (March 2008).

22. FAR Clause **52.242-3, Penalties for Unallowable Costs** (May 2001).

23. FAR Clause **52.243-2, Changes--Cost Reimbursement** (August 1987), **Alternate V** (April 1984).

24. FAR Clause **52.251-1, Government Supply Sources** (April 1984).

b. DEPARTMENT OF HEALTH AND HUMAN SERVICES ACQUISITION REGULATION (HHSAR) (48 CHAPTER 3) CLAUSES:

1. HHSAR Clause **352.223-70, Safety and Health** (January 2006).

2. HHSAR Clause **352.224-70, Confidentiality of Information** (January 2006).

3. HHSAR Clause **352.270-1, Accessibility of Meetings, Conferences and Seminars to Persons with Disabilities** (January 2001).

4. HHSAR Clause **352.270-7, Paperwork Reduction Act** (January 2006).

5. HHSAR Clause **352.333-7001, Choice of Law (Overseas)** (March 2005).

c. *NATIONAL INSTITUTES OF HEALTH (NIH) RESEARCH CONTRACTING (RC) CLAUSES:*

The following clauses are attached and made a part of this contract:

1. ***NIH (RC)-7, Procurement of Certain Equipment*** *(April 1984).*

2. ***NIH(RC)-11, Research Patient Care Costs*** *(4/1/84).*

ARTICLE I.4. ADDITIONAL FAR CONTRACT CLAUSES INCLUDED IN FULL TEXT

This contract incorporates the following clauses in full text.

FEDERAL ACQUISITION REGULATION (FAR)(48 CFR CHAPTER 1)CLAUSES:

a. FAR Clause **52.219-28, Post-Award Small Business Program Rerepresentation** (April 2009).

(a) *Definitions.* As used in this clause--

Long-term contract means a contract of more than five years in duration, including options. However, the term does not include contracts that exceed five years in duration because the period of performance has been extended for a cumulative period not to exceed six months under the clause at 52.217-8, Option to Extend Services, or other appropriate authority.

Small business concern means a concern, including its affiliates, that is independently owned and operated, not dominant in the field of operation in which it is bidding on Government contracts, and qualified as a small business under the criteria in 13 CFR part 121 and the size standard in paragraph (c) of this clause. Such a concern is "not dominant in its field of operation" when it does not exercise a controlling or major influence on a national basis in a kind of business activity in which a number of business concerns are primarily engaged. In determining whether dominance exists, consideration shall be given to all appropriate factors, including volume of business, number of employees, financial resources, competitive status or position, ownership or control of materials, processes, patents, license agreements, facilities, sales territory, and nature of business activity.

(b) If the Contractor represented that it was a small business concern prior to award of this contract, the Contractor shall rerepresent its size status according to paragraph (e) of this clause or, if applicable, paragraph (g) of this clause, upon the occurrence of any of the following:

(1) Within 30 days after execution of a novation agreement or within 30 days after modification of the contract to include this clause, if the novation agreement was executed prior to inclusion of this clause in the contract.

(2) Within 30 days after a merger or acquisition that does not require a novation or within 30 days after modification of the contract to include this clause, if the merger or acquisition occurred prior to inclusion of this clause in the contract.

(3) For long-term contracts--

(i) Within 60 to 120 days prior to the end of the fifth year of the contract; and
(ii) Within 60 to 120 days prior to the date specified in the contract for exercising any option thereafter.

(c) The Contractor shall rerepresent its size status in accordance with the size standard in effect at the time of this rerepresentation that corresponds to the North American Industry Classification System (NAICS) code assigned to this contract. The small business size standard corresponding to this NAICS code can be found at http://www.sba.gov/contractingopportunities/officials/size/index.html.

(d) The small business size standard for a Contractor providing a product which it does not manufacture itself, for a contract other than a construction or service contract, is 500 employees.

(e) Except as provided in paragraph (g) of this clause, the Contractor shall make the rerepresentation required by paragraph (b) of this clause by validating or updating all its representations in the Online Representations and Certifications Application and its data in the Central Contractor Registration, as necessary, to ensure that they reflect the Contractor's current status. The Contractor shall notify the contracting office in writing within the timeframes specified in paragraph (b) of this clause that the data have been validated or updated, and provide the date of the validation or update.

(f) If the Contractor represented that it was other than a small business concern prior to award of this contract, the Contractor may, but is not required to, take the actions required by paragraphs (e) or (g) of this clause.

(g) If the Contractor does not have representations and certifications in ORCA, or does not have a representation in ORCA for the NAICS code applicable to this contract, the Contractor is required to complete the following rerepresentation and submit it to the contracting office, along with the contract number and the date on which the rerepresentation was completed:

The Contractor represents that it [ ] is, [ ] is not a small business concern under NAICS Code assigned to contract number.

[Contractor to sign and date and insert authorized signer's name and title].

(End of clause)

b. FAR Clause **52.222-39, Notification Of Employee Rights Concerning Payment Of Union Dues Or Fees** (December 2004)

(a) Definition. As used in this clause --

United States means the 50 States, the District of Columbia, Puerto Rico, the Northern Mariana Islands, American Samoa, Guam, the U.S. Virgin Islands, and Wake Island.

(b) Except as provided in paragraph (e) of this clause, during the term of this contract, the Contractor shall post a notice, in the form of a poster, informing employees of their rights concerning union membership and payment of union dues and fees, in conspicuous places in and about all its plants and offices, including all places where notices to employees are customarily posted. The notice shall include the following information (except that the information pertaining to National Labor Relations Board shall not be included in notices posted in the plants or offices of carriers subject to the Railway Labor Act, as amended (45 U.S.C. 151-188)).

Notice to Employees

Under Federal law, employees cannot be required to join a union or maintain membership in a union in order to retain their jobs. Under certain conditions, the law permits a union and an employer to enter into a union-security agreement requiring employees to pay uniform periodic dues and initiation fees. However, employees who are not union members can object to the use of their payments for certain purposes and can only be required to pay their share of union costs relating to collective bargaining, contract administration, and grievance adjustment.

If you do not want to pay that portion of dues or fees used to support activities not related to collective bargaining, contract administration, or grievance adjustment, you are entitled to an appropriate reduction in your payment. If you believe that you have been required to pay dues or fees used in part to support activities not related to collective bargaining, contract administration, or grievance adjustment, you may be entitled to a refund and to an appropriate reduction in future payments.

For further information concerning your rights, you may wish to contact the National Labor Relations Board (NLRB) either at one of its Regional offices or at the following address or toll free number:

National Labor Relations Board
Division of Information
1099 14th Street, N.W.
Washington, DC 20570
1-866-667-6572
1-866-316-6572 (TTY)

To locate the nearest NLRB office, see NLRB's website at http://www.nlrb.gov.

(c) The Contractor shall comply with all provisions of Executive Order 13201 of February 17, 2001, and related implementing regulations at 29 CFR part 470, and orders of the Secretary of Labor.

(d) In the event that the Contractor does not comply with any of the requirements set forth in paragraphs (b), (c), or (g), the Secretary may direct that this contract be cancelled, terminated, or suspended in whole or in part, and declare the Contractor ineligible for further Government contracts in accordance with procedures at 29 CFR part 470, Subpart B--Compliance Evaluations, Complaint Investigations and Enforcement Procedures. Such other sanctions or remedies may be imposed as are provided by 29 CFR part 470, which implements Executive Order 13201, or as are otherwise provided by law.

(e) The requirement to post the employee notice in paragraph (b) does not apply to--

(1)Contractors and subcontractors that employ fewer than 15 persons;

(2)Contractor establishments or construction work sites where no union has been formally recognized by the Contractor or certified as the exclusive bargaining representative of the Contractor's employees;

(3) Contractor establishments or construction work sites located in a jurisdiction named in the definition of the United States in which the law of that jurisdiction forbids enforcement of union-security agreements;

(4)Contractor facilities where upon the written request of the Contractor, the Department of Labor Deputy Assistant Secretary for Labor-Management Programs has waived the posting requirements with respect to any of the Contractor's facilities if the Deputy Assistant Secretary finds that the Contractor has demonstrated that--

(i) The facility is in all respects separate and distinct from activities of the Contractor related to the performance of a contract; and

(ii) Such a waiver will not interfere with or impede the effectuation of the Executive order; or

(5) Work outside the United States that does not involve the recruitment or employment of workers within the United States.

(f) The Department of Labor publishes the official employee notice in two variations; one for contractors covered by the Railway Labor Act and a second for all other contractors. The Contractor shall--

(1) Obtain the required employee notice poster from the Division of Interpretations and Standards, Office of Labor-Management Standards, U.S. Department of Labor, 200 Constitution Avenue, NW, Room N-5605, Washington, DC 2021, or from any field office of the Department's Office of Labor-Management Standards or Office of Federal Contract Compliance Programs;

(2) Download a copy of the poster from the Office of Labor-Management Standards website at http://www.olms.dol.gov; or

(3) Reproduce and use exact duplicate copies of the Department of Labor's official poster.

(g) The Contractor shall include the substance of this clause in every subcontract or purchase order that exceeds the simplified acquisition threshold, entered into in connection with this contract, unless exempted by the Department of Labor Deputy Assistant Secretary for Labor-Management Programs on account of special circumstances in the national interest under authority of 29 CFR 470.3(c). For indefinite quantity subcontracts, the Contractor shall include the substance of this clause if the value of orders in any calendar year of the subcontract is expected to exceed the simplified acquisition threshold. Pursuant to 29 CFR part 470, Subpart B--Compliance Evaluations, Complaint Investigations and Enforcement Procedures, the Secretary of Labor may direct the Contractor to take such action in the enforcement of these regulations, including the imposition of sanctions for noncompliance with respect to any such subcontract or purchase order. If the Contractor becomes involved in litigation with a subcontractor or vendor, or is threatened with

such involvement, as a result of such direction, the Contractor may request the United States, through the Secretary of Labor, to enter into such litigation to protect the interests of the United States.

(End of Clause)

PART III - LIST OF DOCUMENTS, EXHIBITS AND OTHER ATTACHMENTS

SECTION J - LIST OF ATTACHMENTS

The following documents are attached and incorporated in this contract:

1. Statement of Work

Statement of Work, dated 30 September 2009

2. Invoice/Financing Request and Contract Financial Reporting Instructions for NIH Cost-Reimbursement Type Contracts, NIH(RC)-4

Invoice/Financing Request and Contract Financial Reporting Instructions for NIH Cost-Reimbursement Type Contracts, NIH(RC)-4, (8/08), 6 pages.

3. Privacy Act System of Records, Number

Privacy Act System of Records, Number 09-25-0087

4. Small Business Subcontracting Plan

Small Business Subcontracting Plan, dated 12/2/2009, 13 pages.

5. Safety and Health

Safety and Health, HHSAR Clause 352.223-70, (1/06), 1 page.

6. Procurement of Certain Equipment

Procurement of Certain Equipment, NIH(RC)-7, 4/1/84, 1 page.

7. Research Patient Care Costs

Research Patient Care Costs, NIH(RC)-11, 4/1/84, 1 page.

8. Disclosure of Lobbying Activities, SF-LLL

Disclosure of Lobbying Activities, SF-LLL, dated 7/97, 3 pages.

9. Government Property - Schedule

Government Property - Schedule ___, dated _____, _ pages.

10. Commitment To Protect Non-Public Information

Commitment To Protect Non-Public Information, 1 page. Located at: http://irm.cit.nih.gov/security/Nondisclosure.pdf

11. Roster of Employees Requiring Suitability Investigations

Roster of Employees Requiring Suitability Investigations, 1 page. Excel file located at: http://ais.nci.nih.gov/forms/Suitability-roster.xls

12. Employee Separation Checklist

Employee Separation Checklist, 1 page. Fillable PDF format located at: http://rcb.cancer.gov/rcb-internet/forms/Emp-sep-checklist.pdf

13. Deliverables

Deliverables

PART IV - REPRESENTATIONS AND INSTRUCTIONS

SECTION K - REPRESENTATIONS AND CERTIFICATIONS

The following documents are incorporated by reference in this contract:

1. Annual Representations and Certifications completed and located at the Online Representations and Certifications Application (ORCA) website. This includes the changes identified in paragraph (b) of the FAR provision 52.204-8, Annual Representations and Certifications, contained in the Contractor's proposal.

2. NIH Representations & Certifications, dated December 8, 2008.

4. Human Subjects Assurance Identification Number FWA00005019.

5. Animal Welfare Assurance Number FWA00002055.

END of the SCHEDULE

(CONTRACT)

Attachment 1 :
Statement of Work

5. STATEMENT OF WORK

1. SCOPE

Because of its high case fatality rate, ability to spread easily by human-human contact, and potential for aerosol release, Lassa virus (LASV), the causative agent of Lassa fever (LF), is classified as a Biosafety Level 4 (BSL-4) and NIAID Biodefense category A agent. The potential use of LASV as a biological weapon directed against civilian or military targets necessitates development of counterthreat measures, such as diagnostic assays, vaccines and therapeutics. The impact of LF in endemic areas of West Africa is immense, and therefore means to diagnose, treat or prevent this viral hemorrhagic fever (VHF) will provide a very significant public health benefit. Our team has produced prototype LASV enzyme-linked immunosorbent assays (ELISA) that are based on recombinant proteins rather than on reagents that must be produced in high containment laboratories. We have also established research programs in Sierra Leone, Guinea and Nigeria that provide unique clinical and laboratory resources for VHF research. The goals of this program are to use these new laboratory and clinical resources to identify novel B cell epitopes on LASV proteins and elucidate mechanisms of antibody-mediated protection or pathogenesis in humans infected with LASV. This project will process in a manner strictly dependent on timely achievement of the following Requirements and Milestones:

a. **EPITOPE IDENTIFICATION**

Milestone 1: Derive human monoclonal antibodies (MAbs) to LASV proteins.

Milestone 2: Map linear or conformational B cell epitopes using mutant and wild-type recombinant LASV proteins, and overlapping synthetic peptides.

Milestone 3: Determine X-ray crystallographic structures of the GP, NP and Z proteins of LASV and other arenaviruses in complex with human MAbs.

Milestone 4. Develop novel screening methods for LASV B cell epitope identification, including deuterium exchange mass spectrometry and small angle X-ray scattering.

b. **EPITOPE VALIDATION**

Milestone 5: Define the kinetics of IgM- and IgG-responses to defined epitopes on GP, NP and Z proteins of LASV using variations of LF ELISA and ELISPOT assays in acutely-infected, convalescent, and previously-exposed persons.

Milestone 6: Quantify and characterize anti-LASV neutralizing and enhancing antibody responses in a well-characterized cohort of persons exposed to diverse strains of LASV at different stages and with different severities of illness.

Milestone 7: Validate protective or pathogenic role of antibodies to defined epitopes on GP, NP and Z proteins of LASV using *in vivo* challenge and protection studies in our well-established guinea pig and non-human primate models of LF.

c. **MECHANISMS OF ANTIBODY PROTECTION AND/OR PATHOGENESIS**.

Milestone 8: Determine LASV sequence diversity and impact on recognition of B cell epitopes by LF patients.

Milestone 9: Evaluate all B cell epitopes discovered (under Requirement a) for functionality by comparing these acquired responses with LF clinical disease outcomes to identify the mechanisms of protection or pathogenesis in humans.

d. **EPITOPE SUBMISSION.**

Milestone 10: As specified in the RFP, submit information on LASV B cell epitopes developed under this contract to the Immune Epitope Database and Analysis Resource (www.ImmuneEpitope.org) to facilitate access and use of these data and tools by the broader research community.

2. TECHNICAL REQUIREMENTS

Independently, and not as an agent of the Government, the Contractor shall furnish all necessary services, qualified professional, technical, and administrative personnel, material, equipment and facilities, not otherwise provided by the Government under the terms of this contract, as needed to perform the tasks set forth below. Specifically, the Contractor shall conduct the following activities:

A. EPITOPE IDENTIFICATION

To guide the rational design of LF immunotherapeutics and vaccines, James E. Robinson, M.D. (Principal Investigator, Tulane) will select, produce and characterize human MAbs reactive with LASV GPC, GP1 and GP2, NP and Z (Milestone 1). Derivation of human MAbs will permit identification of conserved and variant epitopes on this important biothreat agent and public health pathogen. The principle focus will be on epitopes on LASV GP, as these antibodies are most likely to be involved in virus neutralization or enhancement. However, characterization of epitopes on internal proteins NP and Z across divergent isolates of LASV can be valuable for development of diagnostic assays, and will also be pursued. The primary method for generating LASV specific human MAbs will be EBV transformation of B cells isolated from peripheral blood of patients who are infected with LASV or who have survived LASV infection. Biased antibody fragment (Fab) phage display libraries will be used as an alternative approach for identification of human MAbs specific for LASV.

LASV IgG-capture ELISA developed previously by this research team and produced to commercial standards by Corgenix will be used to screen for antibody producing cells. Similar ELISA will be developed for divergent strains of LASV, and for recombinant LASV proteins produced with defined site-directed or other mutations in specific B cell epitopes. We have also developed protocols for producing virus-like particles (VLP). ELISA using VLP potentially can detect conformational antibodies not present on recombinant proteins. Luis M. Branco (Autoimmune) and Erica Ollmann Saphire, Ph.D. (TSRI) have also cloned New World arenavirus virus GPC, GP1, GP2 and NP), which function in ELISA and can be used to screen human MAbs for cross-reactivity amongst divergent arenaviruses or to select for human MAbs specific for these New World arenaviruses.

The human MAbs produced in Milestone 1 will be characterized to determine whether binding occurs on linear vs. conformational epitopes, monomer or oligomer, recombinant proteins vs. VLP, glycosylated or vs. deglycosylated GP or point mutants that delete glycosylation sites. In Milestone 2 Robert F. Garry, Ph.D. (Program Manager, Tulane) will map linear or conformational B cell epitopes using mutant and wild-type recombinant LASV proteins. To determine the topological relationships between epitopes recognized by the human and murine MAb to LASV recombinant GPC, GP1, GP2, NP and Z cross-competition analyses will be performed. These ELISA will be used to determine cross-competition patterns and whether the antibodies recognized overlapping or nonoverlapping sites. Additional definition of the binding specificity of the LASV specific MAb will be determined by binding studies using peptides from LASV proteins or synthetic peptides.

Crystal structures of antibodies in complex with these proteins have illustrated vulnerable epitopes, elucidated key differences between neutralizing and non-neutralizing antibodies when they recognize similar epitopes, and have directed the design of improved immunogens. In Milestone 3 Dr. Ollmann Saphire (TSRI) will determine X-ray crystallographic structures of the GP, NP and Z proteins of LASV and other arenaviruses in complex with MAbs. Dr. Ollmann Saphire, who recently determined the crystal structure of the Ebola virus (EBOV) glycoprotein in its prefusion conformation, have developed advanced strategies for construct refinement and crystal optimization in event of poorly diffracting crystals. The use of multiple constructs and multiple antibodies, screened in parallel on microscale for crystallization, should allow identification of crystallizable complexes. Further, this project will implement and refine novel methods for small-scale expression, purification, nanoscale crystallization screening, and diffraction screening in chips.

B. EPITOPE VALIDATION

The kinetics of IgM- and IgG-responses in LASV acutely-infected, convalescent, and previously-exposed persons for identified epitopes on GP, NP and Z will be defined in Milestone 5 using variations of LF

ELISA and ELISPOT assays. A set of appropriately cloned, mutated and expressed LASV proteins and other cross-reactive proteins (ie. Junin virus proteins) and synthetic peptides assembled under Milestone 2-4, will allow an evaluation of the reactivities to particular epitopes, or a panel of epitopes representative of divergent antigenic sites on the proteins of LASV and other arenaviruses. The analysis of immunodominance patterns of various B cell epitopes on the population level will be crucial for the identification of epitopes that are frequently targeted in a population of individuals with LASV infection.

Studies of passive transfer in LF suggest that neutralizing sera, if correctly selected for LASV strain, can mediate protection against LASV challenge. Thus, neutralizing human MAb could form the basis for a novel LF immunotherapeutic. Studies by team member Thomas W. Geisbert, Ph.D. and others strongly suggest that the production of neutralizing antibodies is synergistic with T cell responses in protection against LASV and other arenaviruses. It is apparent that candidate LF vaccines should be designed to elicit LASV neutralizing antibody responses. In Milestone 6 Dr. Geisbert and colleagues will quantify and characterize anti-LASV neutralizing and enhancing antibody responses in a well-characterized cohort of persons exposed to diverse strains of LASV at different stages and with different severities of illness. The procedures used will also detect enhancing antibodies, if present. The presence or absence of antibody enhancement may have important implications for clinical outcomes of LASV infection, and potentially also could have profound implications for LF vaccine development

Passive transfer of serum or MAbs in animal models has been used extensively to validate the protective or pathogenic roles of antibodies in various VHF. These studies will be extended to human MAb representing B cell epitopes of LASV proteins. In Milestone 7 Dr. Geisbert and co-workers will validate protective or pathogenic role of antibodies to defined epitopes on GP, NP and Z proteins of LASV using *in vivo* challenge and protection studies in well-established guinea pig and non-human primate models of LF. The proposed passive transfer studies of LASV MAbs will identify MAb that function alone and in combination to provide a detectable level of protection in strain 13 guinea pigs and cynomolgus macaques. This information will provide important information on the potential protective roles of these MAbs, and may identify potential immunotherapeutics for the treatment of LF, which could provide additional clinical benefits when used in combination with antiviral drugs such as ribavirin. Alternative approaches to defining the potential role of B cell epitopes in LF involve the use of our previously developed LASV recombinant vaccine. Immunization of *Cynomolgus macaques* with VSVΔG/LVGPC resulted in complete protection from lethal LASV infection. Animals immunized with the construct containing wild-type LASV GP will protect from lethal challenge, but recombinant vaccine with critical B cell epitopes eliminated in LASV GP would not be protective or would only offer partial protection from lethal LASV challenge.

C. MECHANISMS OF ANTIBODY PROTECTION AND/OR PATHOGENESIS.

Kathleen H. Rubins, Ph.D., Pardis C. Sabeti, M.D., P.D. and co-workers will determine LASV sequence diversity and impact on recognition of B cell epitopes by LF patients in studies proposed under Milestone 8. They will provide a complete mapping of variability in viral strains through high-throughput, whole genome sequencing of several hundred LASV strains. Large-scale sequence data and identification of variation in LASV will assist in determining how viral factors influence critical aspects of the human immune response, such as reactivity to a particular LASV epitope, immune responses elicited by specific LASV proteins, or neutralizing Ab responses. The viral genomics data will allow correlations with multiple strain specific differences in virulence, adaptive immune responses.

Through a unique new capacity building project in West Africa initiated by Daniel G. Bausch, M.D., MPH&TM, the study team will have access to clinical samples from Sierra Leone, Guinea and Liberia, with a laboratory for real-time in-country diagnostics and research in Kenema, Sierra Leone. In addition, Drs. Sabeti and Christian Happi provide newly developed clinical capacity for studies of LF in Irrua, Nigeria. Thus, clinical observations and samples from patients across the range of LASV in West Africa will be available for study. The high incidence of LF, along with these infrastructure and resources, offer unique opportunities to conduct detailed and integrated studies of the correlates of protective immunity in a VHF and Category A Select Agent. Correlations will be made between clinical outcomes, including mild, severe or fatal disease outcomes and various B cell epitope responses.

D. EPITOPE SUBMISSION

As specified in the RFP, the collaborative team will submit information on LASV B cell epitopes developed under this contract to the Immune Epitope Database and Analysis Resource (www.ImmuneEpitope.org) to facilitate access and use of these data and tools by the broader research community (Milestone 10). The contractor has all the necessary infrastructure and expertise to prepare and submit data to the Immune Epitope Database and Analysis Resource (IEDB: www.ImmuneEpitope.org) in XML format. The following B cell epitope information shall be submitted to the IEDB:

a. B cell epitopes, including linear and conformational sites, identifiable by their epitope sequence (i.e., amino acid, carbohydrate, or lipid composition for linear and conformational antibody epitopes) ;
b. composition of natural, artificial, and modified amino acids as may occur during post-translational modifications of whole molecules or ligands); and haptens associated with the epitope;
c. pathogen and antigen;
d. disease state or pathogen replication stage in which the epitope is expressed;
e. epitope identification methods;
f. antibody isotype(s) that recognize the epitope;
g. antibody/antigen binding affinity;
h. methods used to test immune recognition of epitopes, such as validation of immunogenicity and/or antigenicity *in vivo* and *in vitro* (e.g., ELISA, ELISPOT, pathogen neutralization, protection studies); and
i. where available, the three-dimensional structure of the antigen from which the epitope derived and the epitope location on the whole antigen.

E. NOVEL METHODS (IF APPLICABLE)

In Milestone 4 Dr. Saphire and Virgil L. Woods, M.D. (UCSD) will develop novel screening methods for LASV B cell epitope identification, including deuterium exchange mass spectrometry (DXMS) and small angle X-ray scattering (SAXS). DXMS is a biophysical technique able to describe solvent exposure and mobility of each region of a protein or protein complex by employing peptide amide hydrogens as water-accessibility probes. One amide hydrogen is present on each amino acid in a protein except proline. Each peptide amide hydrogen continuously and reversibly interchanges with hydrogen present in water, in a manner reflecting the solvent accessibility and thermodynamic stability of the individual peptide bond to which it is attached. By allowing these hydrogens, within the structured protein, to exchange with solvent deuterium (as D_2O), acid pH-quenching the reaction (greatly slowing further exchange), digesting the protein with pepsin, and analyzing the resulting deuterium-exchanged peptides by mass spectrometry, we can determine which amino acids of a protein are exposed to solvent and which are buried. SAXS records the elastic scattering of X-rays at very low angles allowing structural analysis of proteins in solution, without the need for crystals.

DXMS and SAXS analyses will be employed to identify antibody-binding sites on LASV proteins, and provide alternative and complementary sources of information to X-ray crystallography. Our use of the three complementary techniques will ensure that some information can be gained from nearly every sample. SAXS, for example, will provide low-resolution (~10Å, similar to cryoEM) molecular envelopes of antibody-GP complexes. Use of SAXS in this proposal will permit rapid analysis of proteins that do not crystallize or diffract well, proteins for which only a component or truncated versions could be crystallized (for example, using SAXS to model intact GP if only GP1 can be crystallized), or any protein (crystallized or non-crystallized) in its solution state. As a proof of concept, we determined a SAXS-derived structure of EBOV GP in complex with Fab KZ52 prior to growth of diffracting crystals, and we have also modeled an intact mucin-containing EBOV GP, which is refractory to crystallization. DXMS analysis can identify peptide amides present in both linear and conformational LASV protein-antibody binding surfaces and can provide a rapid, provisional definition of candidate LASV protein epitopes to guide definitive linear and combinatorial epitope mapping studies in Milestone 2. We have had remarkable success in our early fragmentation studies of Lassa GP1. Our single-pass, limited tuning efforts to date have already resulted in 75% primary sequence coverage with 155 densely overlapping probe fragments. DXMS will also be used to refine the design of LASV protein crystallographic constructs, employing approaches that we

successfully applied to the redesign of other poorly-crystallizing proteins.

F. SCIENTIFIC, TECHNICAL, MANAGEMENT, AND ADMINISTRATIVE TEAM

Tulane University (Tulane) will provide the central leadership of the proposed Project. James E. Robinson, M.D. will serve as PI, direct the overall research program and perform selection of human monoclonal antibodies to LASV proteins for identification of B cell epitopes. Dr. Robinson has many years of experience in generating human and monkey MAbs that recognize various enveloped viruses, and was the first to explore the identification of conserved and variant epitopes of HIV-1 SU (gp120) using human MAb produced by EBV-transformed cell lines. Robert F. Garry, Ph.D. will serve as Project Manager and supervise ELISA and ELISPOT assay validation of LASV B cell epitopes. Although based in an academic institution, he is well-qualified to served the PM function for this contract, and has managed several large interdisciplinary projects in the past. For example, he managed a subset of the current Project subcontractors, including the private companies (Autoimmune and Corgenix), in a large NIH contract (Cooperative Agreement) that successfully produced prototype LASV recombinant antigen-based ELISA. Drs. Robinson and Garry will be assisted in the management of the proposed Project by an Internal Advisory Board (IAB) consisting of the Directors of the proposed subcontracts.

Erica Ollmann Saphire, Ph.D. (Director, TSRI subcontract) will express LASV recombinant proteins, and perform X-ray crystallographic and other structural studies of LASV proteins (e.g. SAXS) in complex with MAbs to map B cell epitopes. Dr. Ollmann Saphire, who recently solved the crystal structure of the prefusion form of EBOV GP, has also developed a number of additional novel techniques that can provide structural information of antibody binding sites. She will be supported in these efforts by Virgil L. Woods Jr., M.D. (Director, UCSD subcontract) who will perform DXMS for analysis of LASV protein crystallization constructs and antibody:protein interfaces. Thomas W. Geisbert, Ph.D. (Director, NEIDL Institute subcontract) will perform/supervise live arenavirus culture studies, including LASV neutralization and animals studies, in BSL-4 laboratories and perform the passive transfer studies of selected LASV MAbs in guinea pigs and macaques. Kathleen H. Rubins, Ph.D. (Director, Whitehead subcontract) and Pardis C. Sabeti, M.D., Ph.D. (Director, Broad subcontract) will perform high-throughput viral sequencing, genomic analyses, which are critical to defining the role of B cell epitope variation in the pathogenesis of LF.

Members of this research team have established research programs in Sierra Leone, Guinea and Nigeria that provide unique clinical and laboratory resources for VHF research. Daniel G. Bausch, M.D. (Tulane) will oversee the acquisition of clinical samples in Sierra Leone, Guinea, Liberia, and elsewhere, and maintain clinical and laboratory infrastructure at the Lassa Ward of Kenema Government Hospital, Kenema, Sierra Leone. He has extensive experience overseeing and managing field research projects and investigations in sub-Saharan Africa, Latin America, and Asia. Dr. Sheik Humarr Khan, Director of the Lassa Ward at Kenema Government Hospital, Sierra Leone will provide samples from consented patients with suspected Lassa virus infection. Augustine Goba, Ph.D. will manage the laboratory for in-country performance of ELISA and ELISPOT assays. Mamadou Coulibaly, Ph.D. manages Tulane-CIRIT, which will be a major source of samples from consented patients with suspected LASV infection. In collaboration with Dr. Sabeti, Dr. Christian Happi, Irrua Specialist Teaching Hospital, Nigeria will provide samples from consented patients with suspected Lassa virus infection, and manage the laboratory for in country performance of ELISA and ELISPOT assays. Delia Enria, M.D., Instituto National de Enfermedades Virales, Pergamino. Argentina, is an established long-term collaborator who provides a major source of archival samples from patients with suspected New World arenavirus infection.

Our team has produced prototype LASV enzyme-linked immunosorbent assays (ELISA) that are based on recombinant proteins rather than on reagents that must be produced in high containment laboratories. Luis M. Branco (Autoimmune) and AIT staff will provide recombinant LASV NP, GP1 and GP2 for recombinant Lassa fever ELISA and ELISPOT assays, MAb scale-up, and MAb purification and production. F. Jon Geske, Ph.D. and Corgenix will provide Ab-capture ELISA using wild-type and mutant recombinant LASV proteins from divergent isolates, which are essential for B cell epitopes identification, validation and mechanism of action studies.

Consultants with special expertise in support of this project include Mary C. Guttieri, Ph.D. will consult on implementation of innovative methods for LASV-specific antibody production using phage display, and antibody cassette expression system for conversion of phage display-selected Fab fragments into complete human IgG1. Lisa E. Hensley, Ph.D. will provide a back-up performance site for live arenavirus culture studies, including virus neutralization and animals studies, in BSL-4 laboratories at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID).

G. PROJECT MANAGEMENT

The Contractor shall provide for project management as follows:

1. STAFFING, ORGANIZATION, COMMUNICATION, ETC.

The Contractor shall provide all expertise needed for the implementation of the Project to be performed under this contract, including: research, clinical, statistical, management and administrative activities. The Contractor's team shall include strong scientific leadership as well as experience and expertise in the management, design and execution of a multidisciplinary research program. Dr. Robinson, the Principal Investigator (PI) shall be responsible for all aspects of project performance and communication with the Project Officer and the Contracting Officer. In addition, the Contractor shall provide a Project Manager (PM), Dr. Garry, who is responsible for the day-to-day monitoring and tracking of progress and timelines, the coordination of project activities and costs incurred.

All proposed subcontractors provide a subcontract Director (Drs. Saphire, Woods, Geisbert, Rubins, Sabeti, and Geske, and Mr. Branco), who will serve as the primary contacts with the PI, PM, Tulane and its staff during the course of the project. In addition to their scientific and technical contributions to achieving Project Requirements and Milestones, the Project management responsibilities of these subcontract Directors will be to assist in the designing and scheduling of the subcontract and manage timelines and coordinate research activities to insure completion of the project within the allotted time. Efficient analysis, transfer, integration, monitoring, and quality control of data collected from contractor and the various subcontractors will be essential to the Project's success and will be the overall responsibility of the PM. This project will use Rescentris' Collaborative Electronic Research Framework™ (CERF) to manage data and documents generated through the contract including monthly and final progress reports from subcontractors, laboratory notebooks of researchers involved in the proposed work. Tulane will provide the core administrative systems and staff to assist the PI and PM in conducting the financial management and reporting activities of the subcontractors.

2. PROJECT TIMELINES

This project will fulfill each of the four Requirements of the BAA, a. EPITOPE IDENTIFICATION, b. EPITOPE VALIDATION, c. ANTIBODY PROTECTION/PATHOGENESIS, and d. EPITOPE SUBMISSION, during an aggressive 5 year Project period. Requirement a. EPITOPE IDENTIFICATION will be initiated in the first project year, and includes Milestones 1-4. Milestone 1 is to derive human MAbs to LASV proteins and will require 60 months for completion. We expect to identify 4-6 (or more) B cell epitopes on each of the LASV structural proteins, GP1, GP2, NP and Z. Milestone 2,= to map LASV epitopes will be initiated within 10 months of the start of the project and will require 50 months to complete. Detailed epitope mapping of all of the human MAbs derived under Milestone 1 will be provided. Milestone 3 to define X-ray structures of LASV proteins will be initiated within 10 months of the start of the project and will require 50 months to complete. Milestone 4, to develop novel epitope screening methods for discovery of B cell epitopes, will be initiated within 10 months of the start of the project and will require 50 months to complete.

Requirement b. EPITOPE VALIDATION includes Milestones 5-7. Milestone 5, studies of the kinetics of antibody response to B cell epitopes of LASV proteins in persons with LF using ELISA and ELISPOT assays, will be initiated within 10 months of the start of the project and will require 50 months to complete. Milestone 6, studies of neutralizing/enhancing Abs to LASV, will be initiated within 10 months of the start of the project and will require 50 months to complete. Studies to validate identified B cell epitopes by passive transfer studies in guinea pigs and Cynomolgus macaques will be initiated within in the third year of the project and require 36 months to complete.

Requirement c. ANTIBODY PROTECTION/PATHOGENESIS will be initiated in the first project year, and includes Milestones 8 and 9. Impact of LASV genetics on epitopes will be initiated within 10 months of the start of the project and will require 50 months to complete. Epitope functionality/clinical outcomes will be initiated within 10 months of the start of the project and will require 50 months to complete.

Requirement d. EPITOPE SUBMISSION will be initiated in the first project year, and includes Milestone 10. ImmuneEpitope.org submission that will be initiated within 10 months of the start of the project and will require 50 months to complete.

3. CONTRACT INITATION MEETING AND ANNUAL PROGRAM MEETINGS

Members of the proposed Project team will participate in the Contract Initiation meeting and annual meetings. The Contract Initiation meeting will be organized by the NIAID and will take place within three months of contract award. The PI and PM shall participate in planning, organization, and logistical arrangements as specified by NIAID. Project-specific annual meetings will be held in New Orleans at approximately one-year intervals.

4. INPUT FOR THE ESTABLISHMENT OF AN EPITOPE DISCOVERY WORKING GROUP (EDWG).

The contractor will provide the Project Officer within fourteen (14) calendar days of contract award, a list of six (6) recommended leading scientists knowledgeable in the multiple research areas related to the proposed contract. These areas will include:

- Humoral immune responses to viral agents of hemorrhagic fevers.
- Viral sequence diversity and its impact on humoral immune responses
- X-ray crystallography and structural analysis of viral proteins
- Vaccinology
- Immunotherapeutics
- Epitope mapping
- Enzyme-linked immunoassay development, and analysis of
- ELISPOT
- Protein expression and mutagenesis of viral proteins

The composition of the EDWG shall be proposed by the Contractor and shall be subject to approval by the Project Officer prior to distribution of invitations by the Project Officer to the proposed EDWG members. The total number of EDWG members shall not exceed seven (7). After award, the Project Officers for the T cell Epitope Discovery and the B cell Epitope Discovery Programs will decide if individual EDWGs for each program or a single EDWG for both programs will be established.

Attachment 2 :

Invoice/Financing Request and Contract Financial Reporting Instructions for NIH Cost-Reimbursement Type Contracts, NIH(RC)-4

INVOICE/FINANCING REQUEST AND CONTRACT FINANCIAL REPORTING INSTRUCTIONS FOR NIH COST-REIMBURSEMENT CONTRACTS, NIH(RC)-4

Format: Payment requests shall be submitted on the Contractor's self-generated form in the manner and format prescribed herein and as illustrated in the Sample Invoice/Financing Request. Standard Form 1034, Public Voucher for Purchases and Services Other Than Personal, may be used in lieu of the Contractor's self-generated form provided it contains all of the information shown on the Sample Invoice/Financing Request. DO NOT include a cover letter with the payment request.

Number of Copies: Payment requests shall be submitted in the quantity specified in the Invoice Submission Instructions in Section G of the Contract Schedule.

Frequency: Payment requests shall not be submitted more frequently than once every two weeks in accordance with the Allowable Cost and Payment Clause incorporated into this contract. Small business concerns may submit invoices/financing requests more frequently than every two weeks when authorized by the Contracting Officer.

Cost Incurrence Period: Costs incurred must be within the contract performance period or covered by precontract cost provisions.

Billing of Costs Incurred: If billed costs include (1) costs of a prior billing period, but not previously billed, or (2) costs incurred during the contract period and claimed after the contract period has expired, the Contractor shall site the amount(s) and month(s) in which it incurred such costs.

Contractor's Fiscal Year: Payment requests shall be prepared in such a manner that the Government can identify costs claimed with the Contractor's fiscal year.

Currency: All NIH contracts are expressed in United States dollars. When the Government pays in a currency other than United States dollars, billings shall be expressed, and payment by the Government shall be made, in that other currency at amounts coincident with actual costs incurred. Currency fluctuations may not be a basis of gain or loss to the Contractor. Notwithstanding the above, the total of all invoices paid under this contract may not exceed the United States dollars authorized.

Costs Requiring Prior Approval: Costs requiring the Contracting Officer's approval, which are not set forth in an Advance Understanding in the contract, shall be identified and reference the Contracting Officer's Authorization (COA) Number. In addition, the Contractor shall show any cost set forth in an Advance Understanding as a separate line item on the payment request.

Invoice/Financing Request Identification: Each payment request shall be identified as either:

(a) **Interim Invoice/Contract Financing Request:** These are interim payment requests submitted during the contract performance period.

(b) **Completion Invoice:** The completion invoice shall be submitted promptly upon completion of the work, but no later than one year from the contract completion date, or within 120 days after settlement of the final indirect cost rates covering the year in which the contract is physically complete (whichever date is later). The Contractor shall submit the completion invoice when all costs have been assigned to the contract and it completes all performance provisions.

(c) **Final Invoice:** A final invoice may be required after the amounts owed have been settled between the Government and the Contractor (e.g., resolution of all suspensions and audit exceptions).

Preparation and Itemization of the Invoice/Financing Request: The Contractor shall furnish the information set forth in the instructions below. The instructions are keyed to the entries on the Sample Invoice/Financing Request. ***All information must be legible or the invoice will be considered improper and returned to the Contractor.***

(a) **Designated Billing Office Name and Address:** Enter the designated billing office name and address, as identified in the Invoice Submission Instructions in Section G of the Contract Schedule.

(b) **Contractor's Name, Address, Point of Contact, TIN, and DUNS or DUNS+4 Number:** Show the Contractor's name and address exactly as they appear in the contract, along with the name, title, phone number, and e-mail address of the person to notify in the event of an improper invoice or, in the case of payment by method other than Electronic Funds Transfer, to whom payment is to be sent. If the remittance name differs from the legal business name, both names must appear on the invoice. Provide the Contractor's Federal Taxpayer Identification Number (TIN) and Data Universal Numbering System (DUNS) or DUNS+4 number. The DUNS number must

identify the Contractor's name and address exactly as stated in the contract, and as registered in the Central Contractor Registration (CCR) database. If the Contractor does not have a valid TIN or DUNS number, provide the Contractor's Vendor Identification Number (VIN), which appears after the Contractor's name on the face page of the award document. ***[Note: A VIN is assigned to new contracts awarded on or after June 4, 2007, and any existing contract modified to include the VIN number.]*** When an approved assignment of claims has been executed, the Contractor shall provide the same information for the assignee as is required for the Contractor (i.e., name, address, point of contact, TIN, and DUNS number), with the remittance information clearly identified as such.

(c) **Invoice/Financing Request Number:** Each payment request must be identified by a unique invoice number, which can only be used one time regardless of the number of contracts or orders held by an organization. For example, if a contractor has already submitted invoice number 05 on one of its contracts or orders, it cannot use that same invoice number on any other contract or order. Payment requests with duplicate invoice numbers will be considered improper and will be returned to the contractor.

The NIH does not prescribe a particular numbering format but suggests using a job or account number for each contract and order followed by a sequential invoice number (example: 8675309-05). Invoice numbers are limited to 30 characters. There are no restrictions on the use of special characters, such as colons, dashes, forward slashes, or parentheses.

If all or part of an invoice is suspended and the contractor chooses to reclaim those costs on a supplemental invoice, the contractor may use the same unique invoice number followed by an alpha character, such as "R" for revised (example: 8675309-05R).

(d) **Date Invoice/Financing Request Prepared:** Insert the date the payment request is prepared.

(e) **Contract Number and Order Number (if applicable):** Insert the contract number and order number (if applicable).

(f) **Effective Date:** Insert the effective date of the contract or if billing under an order, the effective date of the order.

(g) **Total Estimated Cost of Contract/Order:** Insert the total estimated cost of the contract, exclusive of fixed-fee. If billing under an order, insert the total estimated cost of the order, exclusive of fixed-fee. For incrementally funded contracts/orders, enter the amount currently obligated and available for payment.

(h) **Total Fixed-Fee:** Insert the total fixed-fee (where applicable). For incrementally funded contracts/orders, enter the amount currently obligated and available for payment.

(i) **Two-Way/Three-Way Match:** Identify whether payment is to be made using a two-way or three-way match. To determine required payment method, refer to the Invoice Submission Instructions in Section G of the Contract Schedule.

(j) **Office of Acquisitions:** Insert the name of the Office of Acquisitions, as identified in the Invoice Submission Instructions in Section G of the Contract Schedule.

(k) **Central Point of Distribution:** Insert the Central Point of Distribution, as identified in the Invoice Submission Instructions in Section G of the Contract Schedule.

(l) **Billing Period:** Insert the beginning and ending dates (month, day, and year) of the period in which costs were incurred and for which reimbursement is claimed.

(m) **Amount Billed - Current Period:** Insert the amount claimed for the current billing period by major cost element, including any adjustments and fixed-fee. If the Contract Schedule contains separately priced line items, identify the contract line item(s) on the payment request and include a separate breakdown (by major cost element) for each line item.

(n) **Amount Billed - Cumulative:** Insert the cumulative amounts claimed by major cost element, including any adjustments and fixed-fee. If the Contract Schedule contains separately priced line items, identify the contract line item(s) on the payment request and include a separate breakdown (by major cost element) for each line item.

(o) **Direct Costs:** Insert the major cost elements. For each element, consider the application of the paragraph entitled "Costs Requiring Prior Approval" on page 1 of these instructions.

1) **Direct Labor:** Include salaries and wages paid (or accrued) for direct performance of the contract.

   For Level of Effort contracts only, the Contractor shall provide the following information on a separate sheet of paper attached to the payment request:

   - hours or percentage of effort and cost by labor category (as specified in the Level of Effort Article in Section F of the contract) for the current billing period, and

   - hours or percentage of effort and cost by labor category from contract inception through the current billing period. (NOTE: The Contracting Officer may require the Contractor to provide additional breakdown for direct labor, such as position title, employee name, and salary or hourly rate.)

2) **Fringe Benefits:** List any fringe benefits applicable to direct labor and billed as a direct cost. Cite the rate(s) used to calculate fringe benefit costs, if applicable.

3) **Accountable Personal Property:** Include permanent research equipment and general purpose equipment having a unit acquisition cost of $1,000 or more, with a life expectancy of more than two years, and sensitive property regardless of cost (see the HHS *Contractor's Guide for Control of Government Property*). Show permanent research equipment separate from general purpose equipment.

   On a separate sheet of paper attached to the payment request, list each item for which reimbursement is requested. An asterisk (*) shall precede the item if the equipment is below the $1 ,000 approval level. Include reference to the following (as applicable):

   - item number for the specific piece of equipment listed in the Property Schedule, and
   - COA number, if the equipment is not covered by the Property Schedule.

   The Contracting Officer may require the Contractor to provide further itemization of property having specific limitations set forth in the contract.

4) **Materials and Supplies:** Include equipment with unit costs of less than $1 ,000 or an expected service life of two years or less, and consumable material and supplies regardless of amount.

5) **Premium Pay:** List remuneration in excess of the basic hourly rate.

6) **Consultant Fee:** List fees paid to consultants. Identify consultant by name or category as set forth in the contract or COA, as well as the effort (i.e., number of hours, days, etc.) and rate billed.

7) **Travel:** Include domestic and foreign travel. Foreign travel is travel outside of Canada, the United States and its territories and possessions. However, for an organization located outside Canada, the United States and its territories and possessions, foreign travel means travel outside that country. Foreign travel must be billed separately from domestic travel.

8) **Subcontract Costs:** List subcontractor(s) by name and amount billed.

9) **Other:** List all other direct costs in total unless exceeding $1 ,000 in amount. If over $1 ,000, list cost elements and dollar amounts separately. If the contract contains restrictions on any cost element, that cost element must be listed separately.

(p) **Cost of Money (COM):** Cite the COM factor and base in effect during the time the cost was incurred and for which reimbursement is claimed.

(q) **Indirect Costs:** Identify the indirect cost base (IDC), indirect cost rate, and amount billed for each indirect cost category.

(r) **Fixed-Fee:** Cite the formula or method of computation for fixed-fee, if applicable. The fixed-fee must be claimed as provided for by the contract.

(s) **Total Amounts Claimed:** Insert the total amounts claimed for the current and cumulative periods.

(t) **Adjustments:** Include amounts conceded by the Contractor, outstanding suspensions, and/or disapprovals subject to appeal.

(u) **Grand Totals**

(v) **Certification of Salary Rate Limitation:** If required by the contract (see Invoice Submission Instructions in Section G of the Contract Schedule), the Contractor shall include the following certification at the bottom of the payment request:

"I hereby certify that the salaries billed in this payment request are in compliance with the Salary Rate Limitation Provisions in Section H of the contract."

The Contracting Officer may require the Contractor to submit detailed support for costs claimed on one or more interim payment requests.

FINANCIAL REPORTING INSTRUCTIONS:

These instructions are keyed to the Columns on the sample invoice/financing request.

Column A - Expenditure Category: Enter the expenditure categories required by the contract.

Column B - Cumulative Percentage of Effort/Hrs. - Negotiated: Enter the percentage of effort or number of hours agreed to for each employee or labor category listed in Column A.

Column C - Cumulative Percentage of Effort/Hrs. - Actual: Enter the percentage of effort or number of hours worked by each employee or labor category listed in Column A.

Column D - Amount Billed - Current: Enter amounts billed during the current period.

Column E - Amount Billed - Cumulative: Enter the cumulative amounts to date.

Column F - Cost at Completion: Enter data only when the Contractor estimates that a particular expenditure category will vary from the amount negotiated. Realistic estimates are essential.

Column G - Contract Amount: Enter the costs agreed to for all expenditure categories listed in Column A.

Column H - Variance (Over or Under): Show the difference between the estimated costs at completion (Column F) and negotiated costs (Column G) when entries have been made in Column F. This column need not be filled in when Column F is blank. When a line item varies by plus or minus 10 percent, i.e., the percentage arrived at by dividing Column F by Column G, an explanation of the variance should be submitted. In the case of an overrun (net negative variance), this submission shall not be deemed as notice under the Limitation of Cost (Funds) Clause of the contract.

Modifications: Any modification in the amount negotiated for an item since the preceding report should be listed in the appropriate cost category.

Expenditures Not Negotiated: An expenditure for an item for which no amount was negotiated (e.g., at the discretion of the Contractor in performance of its contract) should be listed in the appropriate cost category and all columns filled in, except for G. Column H will of course show a 100 percent variance and will be explained along with those identified under H above.

SAMPLE INVOICE/FINANCING REQUEST AND CONTRACT FINANCIAL REPORT

(a) Designated Billing Office Name and Address:

National Institutes of Health
Office of Financial Management
Commercial Accounts
2115 East Jefferson Street, Room 4B432, MSC 8500
Bethesda, MD 20892-8500

(b) Contractor's Name, Address, Point of Contact, TIN, and DUNS or DUNS+4 Number:

ABC CORPORATION
100 Main Street
Anywhere, U.S.A. Zip+4

Name, Title, Phone Number, and E-mail Address of person to notify in the event of an improper invoice or, in the case of payment by method other than Electronic Funds Transfer, to whom payment is to be sent.

*DUNS or DUNS+4: _____
*TIN: _____

*Provide VIN only if Contractor does not have a valid TIN or DUNS number.

(c) Invoice/Financing Request No.: _____

(d) Date Invoice Prepared: _____

(e) Contract No. and Order No. (if applicable): _____

(f) Effective Date: _____

(g) Total Estimated Cost of Contract/Order: _____

(h) Total Fixed Fee (if applicable): _____

(i) Two-Way Match: ____
Three-Way Match: ____

(j) Office of Acquisitions: _____

(k) Central Point of Distribution: _____

(l) This invoice/financing request represents reimbursable costs for the period from _____ to _____

	Cumulative Percentage of Effort/Hrs		Amount Billed				
Expenditure Category* A	Negotiated B	Actual C	(m) Current D	(n) Cumulative E	Cost at Completion F	Contract Value G	Variance H
(o) Direct Costs:							
(1) Direct Labor							
(2) Fringe Benefits ___%							
(3) Accountable Property							
(4) Materials & Supplies							
(5) Premium Pay							
(6) Consultant Fees							
(7) Travel							
(8) Subcontracts							
(9) Other							
Total Direct Costs							
(p) Cost of Money ___%							
(q) Indirect Costs ___%							
(r) Fixed Fee ___%							
(s) Total Amount Claimed							
(t) Adjustments							
(u) Grand Totals							

"I certify that all payments requested are for appropriate purposes and in accordance with the contract."

_____ _____
(Name of (Title)

*Attach details as specified in the contract or requested by the Contracting Officer

Attachment 3 :
Privacy Act System of Records, Number

Privacy Act Systems of Records Notices (SORNs) or System Notices

There are three types of System Notices that can be cited to cover record systems: Internal, Government and Central.

Internal Notices:

1. Owned by each federal agency to cover their internal records (e.g., HHS, NIH and other OPDIVs);
2. Often referred to as "umbrella" system notices;
3. National Institutes of Health SORNs begin with 09-25-xxxx; and
4. Department of Health & Human Services SORNs begin 09-90-xxxx.

Government Notices:

1. All federal agencies may use these notices to cover government-wide record systems (e.g., OPM, OGE, EEOC, FEMA, GSA, etc.);
2. The physical records contained within the record system belong to the respective federal agency;
3. OPM still retains some authority over the records, e.g., during an appeal process; and
4. Notices begin with GOVT-1, 2, etc.

Central Notices:

1. Owned by OPM who maintains full responsibility for the central record systems (e.g., Personnel Investigations Records);
2. Federal agencies are permitted to maintain copies; and
3. Notices begin with CENTRAL-1, 2, etc.

Listed below are the Privacy Act Systems of Records Notices most commonly referenced within NIH for its record systems:

NIH Internal Systems

09-25-0005, Administration: Library Operations and NIH Library User I.D. File, HHS/NIH

09-25-0007, Administration: NIH Safety Glasses Issuance Program, HHS/NIH/ORS

09-25-0011, Clinical Research: Blood Donor Records, HHS/NIH/CC

09-25-0012, Clinical Research: Candidate Healthy Volunteer Records, HHS/NIH/CC

09-25-0014, Clinical Research: Student Records, HHS/NIH/OD/OIR/OE

09-25-0033, International Activities: Fellowships Awarded by Foreign Organizations, HHS/NIH/FIC

09-25-0034, International Activities: Scholars-in-Residence Program, HHS/NIH/FIC

09-25-0036, Extramural Awards and Chartered Advisory Committees (IMPAC 2), Contract Information (DCIS), and Cooperative Agreement Information, HHS/NIH

09-25-0041, Research Resources: Scientists Requesting Hormone Distribution, HHS/NIH/NIDDK

09-25-0054, Administration: Property Accounting (Card Key System) HHS/NIH/ORS

09-25-0078, Administration: Consultant File, HHS/NIH/NHLBI

09-25-0087, Administration: Senior Staff, HHS/NIH/NIAID

09-25-0099, Clinical Research: Patient Medical Records, HHS/NIH/CC

09-25-0105, Administration: Health Records of Employees, Visiting Scientists, Fellows, and Others Who Receive Medical Care Through the Employee Health Unit, HHS/NIH/ORS

09-25-0106, Administration: Office of the NIH Director and Institute/Center Correspondence Records, HHS/NIH/OD

09-25-0108, Personnel: Guest Researchers, Special Volunteers, and Scientists Emeriti, HHS/NIH/OHRM

09-25-0115, Administration: Curricula Vitae of Consultants and Clinical Investigators, HHS/NIH/NIAID

09-25-0118, Contracts: Professional Services Contractors, HHS/NIH/NCI

09-25-0121, International Activities: Senior International Fellowships Program, HHS/NIH/FIC

09-25-0124, Administration: Pharmacology Research Associates, HHS/NIH/NIGMS

09-25-0140, International Activities: International Scientific Researchers in Intramural Laboratories at the National Institutes of Health, HHS/NIH/FIC/ORS/DIRS

09-25-0156, Records of Participants in Programs and Respondents in Surveys Used to Evaluate Programs of the Public Health Service, HHS/PHS/NIH/OD

09-25-0158, Administration: Records of Applicants and Awardees of the NIH Intramural Research Training Awards Program, HHS/NIH/OD/OIR/OE

09-25-0160, United States Renal Data System (USRDS), HHS/NIH/NIDDK

09-25-0165, National Institutes of Health (NIH) Office of Loan Repayment and Scholarship (OLRS) Records System, HHS/NIH/OD

09-25-0166, Administration: Radiation and Occupational Safety and Health Management Information Systems, HHS/NIH/ORS

09-25-0167, National Institutes of Health (NIH) TRANSHARE Program, HHS/NIH/OD

09-25-0168, Invention, Patent, and Licensing Documents Submitted to the Public Health Service by its Employees, Grantees, Fellowship Recipients, and Contractors, HHS/NIH/OD **[revised 10/3/06]**

09-25-0169, Medical Staff-Credentials Files, HHS/NIH/CC

09-25-0200, Clinical, Basic and Population-based Research Studies of the National Institutes of Health (NIH), HHS/NIH/OD

09-25-0202, Patient Records on PHS Beneficiaries (1935-1974) and Civilly Committed Drug Abusers (1967-1976) Treated at the PHS Hospitals in Fort Worth, Texas, or Lexington, Kentucky, HHS/NIH/NIDA

09-25-0203, National Institute on Drug Abuse, Intramural Research Program, Federal Prisoner and Non-Prisoner Research Files, HHS/NIH/NIDA

09-25-0207, Subject-Participants in Pharmacokinetic Studies on Drugs of Abuse and on Treatment Medications, HHS/NIH/NIDA

09-25-0208, Drug Abuse Treatment Outcome Study (DATOS), HHS/NIH/NIDA

09-25-0209, Subject-Participants in Drug Abuse Research Studies on Drug Dependence and in Research Supporting Investigational New Drug and New Drug Applications, HHS/NIH/NIDA

09-25-0210, Shipment Records of Drugs of Abuse to Authorized Researchers, HHS/NIH/NIDA

09-25-0211, Intramural Research Program Records of In-and Out-Patients with Various Types of Alcohol Abuse and Dependence, Relatives of Patients with Alcoholism, and Healthy Volunteers, HHS/NIH/NIAAA

09-25-0213, Administration: Employee Conduct Investigative Records, HHS/NIH/OD/OM/OA/OMA

09-25-0216, Administration: NIH Electronic Directory, HHS/NIH (to be renamed NIH Enterprise Directory, and amended to include a proposed new use for emergency notification purposes.)

09-25-0217, NIH Business System (NBS), HHS/NIH

HHS Internal Systems

09-90-0008, Conflict of Interest Records, HHS/OS/ASPER

09-90-0018, Personnel Records in Operating Offices, HHS/OS/ASPER

09-90-0020, Suitability for Employment Records, HHS/OS/ASPER (to be renamed HHS Personnel Security, and amended to be compliant with the new I.D. badge procedure under HSPD-12.)

09-90-0024, Unified Financial Management System, HHS/OS

09-90-0039, National Disaster Claims Processing System

09-90-0777, Identification and Credentialing Issuance Station and System, HHS/OCID (in draft form - to cover personal identity verification (PIV) card holders as well as short-term employees, temporary guests and visitors.)

Federal Government Systems

OGE/GOVT-1, Executive Branch Personnel Public Financial Disclosure Reports and Other Name-Retrieved Ethics Program Records

OGE/GOVT-2, Executive Branch Confidential Financial Disclosure Reports

OPM/GOVT-1, General Personnel Records

National Science Foundation (NSF)

NSF-6, Doctorate Records File

NSF-43, Doctorate Work History File

Attachment 4 :
Small Business Subcontracting Plan

SIGNATURE PAGE

Signatures Required:

This subcontracting plan was submitted by:

Signature: Personnel

Typed Name:

Title: Director, Central Procurement Services

Date: 10/20/2009

This plan was reviewed by:

Signature: Personnel

Typed Name:

Title: Contracting Officer Date: 17 Dec 09

This plan was reviewed by:

Signature: Personnel

Typed Name:

Title: HHS Small Business Specialist (SBS) Date: 12/23/09

This plan was reviewed by:

Signature:

Typed Name: PCR RESERVES THE RIGHT TO REVIEW

Title: Small Business Administration Procurement Center Representative

Date:

OFFICE OF SMALL AND DISADVANTAGED BUSINESS UTILIZATION
SMALL BUSINESS SUBCONTRACTING PLAN

HHS Operating Division (OPDIV): National Institutes of Health

DATE OF PLAN: 8-17-09

CONTRACTOR: Tulane University

ADDRESS: 1430 Tulane Avenue, New Orleans, LA70112

DUNN & BRADSTREET NUMBER: 05-378-5812

SOLICITATION OR CONTRACT NUMBER: RFP No. BAA-NIAID-DAIT-NIHAI2008031

ITEM/SERVICE (Description):

The offeror is proposing to discover novel B cell epitopes of Lassa virus (LASV) protein antigens and to elucidate mechanisms of antibody-mediated protection or pathogenesis.

NEW/INITIAL CONTRACT

PERIOD OF CONTRACT PERFORMANCE (Month, Day & Year): 09-15-09 – 09-15-14

Base	$ 15,224,927	Performance Period/Quantity
Option 1:	$	Performance Period/Quantity
Option 2:	$	Performance Period/Quantity
Option 3:	$	Performance Period/Quantity
Option 4:	$	Performance Period/Quantity

$ 15,224,927 Total Contract Cost

CONTRACT MODIFICATION (if applicable)

PERIOD OF CONTRACT PERFORMANCE (Month, Day & Year):_____

Original/Base $_____ Performance Period/Quantity _____
Modification $_____ Performance Period/Quantity _____
Task Order $_____ Performance Period/Quantity _____

$_____ Modified Total Contract Cost

The following outline meets the minimum requirements of section 8(d) of the Small Business Act, as amended, and implemented by Federal Acquisition Regulations (FAR) Subpart 19.7. While this outline has been designed to be consistent with statutory and regulatory requirements, other formats of a subcontracting plan may be acceptable. **It is not intended to replace any existing corporate/commercial plan that is more extensive.**

Failure to include the essential information of FAR Subpart 19.7 may be cause for either a delay in acceptance or the rejection of a bid or offer when a subcontracting plan is required. "SUBCONTRACT," as used in this clause, means any agreement (other than one involving an employer-employee relationship) entered into by a Federal Government prime contractor or subcontractor requesting supplies or services required for performance of the contract or subcontract.

If assistance is needed to locate small business sources, contact the OPDIV Small Business Specialist (SBS) at (_____) _____-_____, the Office of Small and Disadvantage Business Utilization (OSDBU) at (202) 690-7300, or visit the OSDBU website (http://www.hhs.gov/osdbu/staff.html). Also, sources may be obtained through the Central Contractor Registration (http://www.ccr.gov/) website.

Please note that the U.S. Department of Health and Human Services (HHS) has subcontracting goals of 40 % for small business, including Alaska Native Corporations (ANC) and Indian Tribes (hereafter referred to as SB) , 5 % for small disadvantaged business, including Alaska Native Corporations (ANC) and Indian Tribes (hereafter referred to as SDB), 5 % for women-owned business and economically disadvantaged women-owned business (hereafter referred to as WOSB), 3 % for HubZone business (HUBZone) and 3 % service disabled veteran-owned small business (SDVOSB) concerns for fiscal year 2009 . For this procurement, HHS expects all proposed subcontracting plans to contain the following small business goals, a minimum, 30 % for total SB, 11 % for SDB, 5 % for WOSB, 3 % for HubZone and 3 % for SDVOSB concerns. These percentages shall be expressed as percentages of the total estimated subcontracting dollars. **The offeror is required to include an explanation for a category that has zero as a goal.**

1. Type of Plan (check one)

X ***Individual plan*** (all elements developed specifically for this contract and applicable for the full term of this contract).

_____ ***Master plan*** (goals developed for this contract) all other elements standardized and approved by a lead agency Federal Official; must be renewed every three years and contractor must provide copy of lead agency approval.
_____ ***Commercial products/service plan*** (goals are negotiated with the initial agency on a company-wide basis rather than for individual contracts) this plan applies to the entire production of commercial service or items or a portion thereof. The contractor sells commercial products and services customarily used for non-government purposes. The plan is effective during the offeror's fiscal year. ***The contractor must provide a copy of the initial agency approval and must enter an annual SSR into the electronic Subcontracting Reporting System (eSRS) with a breakout of subcontracting prorated for HHS and other Federal agencies.***

2. Goals

Below indicate the dollar and percentage goals for Small Business, including Alaska Native Corporations and Indian Tribes (SB), Small Disadvantaged (SDB), Woman-owned and Economically Disadvantaged Women-Owned (WOSB), Historically Underutilized Business Zone (HUBZone), Service-Disabled Veteran-owned (SDVOSB) small businesses and "Other than small business" (Other) as subcontractors. Indicate the base year and each option year, as specified in FAR 19.704 or project annual subcontracting base and goals under commercial plans.

a. Total estimated dollar value of ALL planned subcontracting, i.e., with ALL types of concerns under this contract is _Subcontractor Info_ (Base Year).

FY _1st Option	FY __2nd Option	FY __3rd Option	FY __4th Option
$ ___	$ _____	$ _____	$ _____

b. Total estimated dollar value and percent of planned subcontracting with SMALL BUSINESSES (including SDB, WOSB, HUBZone and SDVOSB): (% of "a")
$ _Subcontractor Info_ and _____30_____% (Base Year)

FY __1st Option	FY __2nd Option	FY __3rd Option	FY __4th Option
$ _____	$ _____	$ _____	$ _____

c. Total estimated dollar value and percent of planned subcontracting with SMALL DISADVANTAGED BUSINESSES: (% of "a") $ _Subcontractor Info_ and _____11_____% (Base Year).

FY __1st Option	FY __2nd Option	FY __3rd Option	FY __4th Option
$ _____	$ _____	$ _____	$ _____

d. Total estimated dollar value and percent of planned subcontracting with WOMAN-OWNED SMALL BUSINESSES: (% of "a") $ ___Subcontractor Info___ and _____5_____% (Base Year)

FY __1st Option FY __2nd Option FY __3rd Option FY __4th Option

$ ___ $ _____ $ _____ $ _____

e. Total estimated dollar and percent of planned subcontracting with HUBZone SMALL BUSINESSES:
(% of "a") $ ___Subcontractor Info___ and _____3_____% (Base Year)

FY __1st Option FY __2nd Option FY __3rd Option FY __4th Option

$ _____ $ _____ $ _____ $ _____

f. Total estimated dollar and percent of planned subcontracting with SERVICE-DISABLED VETERAN-OWNED SMALL BUSINESSES: (% of "a") ___Subcontractor Info___ and _____3_____% (Base Year)

FY __1st Option FY __2nd Option FY __3rd Option FY __4th Option

$ _____ $ _____ $ _____ $ _____

g. Total estimated dollar and percent of planned subcontracting with "OTHER THAN SMALL BUSINESSES"
(% of "a") $ ___Subcontractor Info___ and _____70_____% (Base Year)

FY __1st Option FY __2nd Option FY __3rd Option FY __4th Option

$ _____ $ _____ $ _____ $ _____

Notes:

1. Federal prime contract goals are:

   SB equals _30_%; SDB equals _11_%; WOSB equals _5_%; HUBZone equals_3_; and SDVOSB equals _3_% may serve as objectives for subcontracting goal development.

2. SDB, WOSB, HUBZone and SDVOSB goals are subsets of SB and should be counted and reported in multiple categories, as appropriate.

3. If any contract has more four options, please attach additional sheets showing dollar amounts and percentages.

Provide a description of ALL the products and/or services to be subcontracted under this contract, and indicate the size and type of business supplying them (check all that apply):

	Products and/or Services	Other	Small Business	SDB	WOSB	Hubz	SDVOSB
1	Protein production		x				
2	Monoclonal antibody production		x				
3	ELISA Production		x				
4	Animal challenges/virology	x					
5	viral sequencing	x					
6	x-ray crystallography, protein structures	x					
7	Research supplies		x	x	x	x	x
8	chemicals		x	x	x	x	x
9	Travel		x	x			
10	Scientific equipment		x	x	x	x	x

i. Provide a description of the method used to develop the subcontracting goals for SB, SDB, WOSB, HUBZone and SDVOSB concerns. Address efforts made to ensure that maximum practicable subcontracting opportunities have been made available for those concerns and explain the method used to identify potential sources for solicitation purposes. Explain the method and state the quantitative basis (in dollars) used to establish the percentage goals. Also, explain how the areas to be subcontracted to SB, WOSB, HUBZone and SDVOSB concerns were determined, how the capabilities of these concerns were considered contract opportunities and how such data comports with the cost proposal. Identify any source lists or other resources used in the determination process. (Attach additional sheets, if necessary.)

_____

j. Indirect costs have _____ have not _X_ been included in the dollar and percentage subcontracting goals above (check one).

k. If indirect costs have been included, explain the method used to determine the proportionate share of such costs to be allocated as subcontracts to SB, SDB, WOSB, HUBZone and SDVOSB concerns:

_____

3. Program Administrator:

NAME/TITLE: _____ Personnel _____

ADDRESS: _____ _____

_____ _____

TELEPHONE: _____ _____

E-MAIL: _____ _____

Duties: Does the individual named above have general overall responsibility for the company's subcontracting program, i.e., developing, preparing, and executing subcontracting plans and monitoring performance relative to the requirements of those subcontracting plans and perform the following duties? (If NO is checked, please who in the company performs those duties, or indicate why the duties are not performed in your company on a separate sheet of paper and submit with the proposed subcontracting plan.)

a. Developing and promoting company-wide policy initiatives that demonstrate the company's support for awarding contracts and subcontracts to SB, SDB, WOSB, HUBZone and SDVOSB concerns; and for assuring that these concerns are included on the source lists for solicitations for products and services they are capable of providing. _X_ yes _____ no

b. Developing and maintaining bidder source lists of SB, SDB, WOSB, HUBZone and SDVOSB concerns from all possible sources; ___X___ yes _____ no

c. Ensuring periodic rotation of potential subcontractors on bidder's lists; _X_ yes ___ no

d. Assuring that SB, SDB, WOSB, HUBZONE and SDVOSB businesses are included on the bidders' list for every subcontract solicitation for products and services that they are capable of providing. ___X__ yes _____ no

e. Ensuring that Requests for Proposals (RFPs) are designed to permit the maximum practicable participation of SB, SDB, WOSB, HUBZone and SDVOSB concerns. _X_ yes _____ no

f. Reviewing subcontract solicitations to remove statements, clauses, etc., which might tend to restrict or prohibit small, 8(a), SDB, WOSB, Hubz and SDVOSB small business participation. ___X__ yes _____ no

g. Accessing various sources for the identification of SB, SDB, WOSB, HUBZone and SDVOSB concerns to include the Central Contractor Registration (http://www.ccr.gov/), local small business and minority associations, local chambers of commerce and Federal agencies' Small Business Offices; _X_ yes ___ no

h. Establishing and maintaining contract and subcontract award records;
___X___ yes _____ no

i. Participating in Business Opportunity Workshops, Minority Business Enterprise Seminars, Trade Fairs, Procurement Conferences, etc; ___X___ yes _____ no

j. Ensuring that SB, SDB, WOSB, HUBZone and SDVOSB concerns are made aware of subcontracting opportunities and assisting concerns in preparing responsive bids to the company; ___X___ yes _____ no

k. Conducting or arranging for the conduct of training for purchasing personnel regarding the intent and impact of Section 8(d) of the Small Business Act, as amended; ___X___ yes _____ no

l. Monitoring the company's subcontracting program performance and making any adjustments necessary to achieve the subcontract plan goals;
___X___ yes _____ no

m. Preparing and submitting timely, required subcontract reports;
___X___ yes _____ no

n. Conducting or arranging training for purchasing personnel regarding the intent and impact of 8(d) of the Small Business Act on purchasing procedures; ___X___ yes _____ no

o. Coordinating the company's activities during the conduct of compliance reviews by Federal agencies; and ___X___ yes _____ no

p. Other duties: _____

4. Equitable Opportunity

Describe efforts the offeror will undertake to ensure that SB, SDB, WOSB, HUBZone and SDVOSB concerns will have an equitable opportunity to compete for subcontracts. These efforts include, but are not limited to, the following activities:

a. Outreach efforts to obtain sources:

1. Subcontractor Info

b. Subcontractor Info

Additional efforts: see below

5. Flow Down Clause

The contractor agrees to include the provisions under FAR 52.219-8, "Utilization of Small Business Concerns," in all acquisitions exceeding the simplified acquisition threshold that offers further subcontracting opportunities. All subcontractors, except small business concerns, that receive subcontracts in excess of $550,000 ($1,000,000 for construction) must adopt and comply with a plan similar to the plan required by FAR 52.219-9, "Small Business Subcontracting Plan." ***Note:*** In accordance with FAR 52.212-5(e) and 52.244-6(c) the contractor is not required to include flow-down clause FAR 52.219.-9 if it is subcontracting commercial items.

6. Reporting and Cooperation

The contractor gives assurance of (1) cooperation in any studies or surveys that may be required; (2) submission of periodic reports which show compliance with the subcontracting plan; (3) submission of its Individual Subcontracting Report (ISR) and Summary Subcontract Report (SSR); and (4) ensuring that subcontractors agree to submit ISRs and SSRs. **The ISR and SSR shall be submitted via the Electronic Subcontracting Reporting System (eSRS) website https://esrs.symplicity.com/index?_tab=signin&cck=1**

Reporting Period	Report Due	Due Date
Oct 1 - Mar 31	ISR	4/30
Apr 1 - Sept 30	ISR	10/30
Oct 1 - Sept 30	SSR	10/30
Contract Completion	OF 312	30 days after completion

See FAR 19.7 for instruction concerning the submission of a Commercial Plan: SSR is due on 10/30 each year for the previous fiscal year ending 9/30.

a. Submit ISR (bi-annually) for the awarding Contracting Officer's review and acceptance via the eSRS website.

b. Currently, SSR (annually) must be submitted for the HHS eSRS Agency Coordinator review and acceptance via the eSRS website. (***Note:*** Log onto the OSDBU website to view the HHS Agency Coordinator contact information (http://www.hhs.gov/osdbu/staff.html).

Note: Due to the nature and complexity of many HHS contracts, the contractor may not be required to submit its subcontracting reports through the eSRS. The HHS Agency Coordinator will confirm the contractor's submission requirements. If the contractor's is required to submit paper copies, it will submit a copy to the HHS Agency Coordinator, the Contracting Officer and the appropriate SBA Commercial Market representative.

7. Record keeping

FAR 19.704(a) (11) requires a list of the types of records your company will maintain to demonstrate the procedures adopted to comply with the requirements and goals in the subcontracting plan. The following is a recitation of the types of records the contractor will maintain to demonstrate the procedures adopted to comply with the requirements and goals in the subcontracting plan. These records will include, but not be limited to, the following:

a. SB, SDB, WOSB, HUBZone and SDVOSB source lists, guides and other data identifying such vendors;

b. Organizations contacted in an attempt to locate SB, SDB, WOSB, HUBZone and SDVOSB sources;

c. On a contract-by-contract basis, records on all subcontract solicitations over $100,000, which indicate for each solicitation (1) whether SB, SDB, WOSB, HUBZone and/or SDVOSB concerns were solicited, if not, why not and the reasons solicited concerns did not receive subcontract awards;

d. Records to support other outreach efforts, e.g., contacts with minority and small business trade associations, attendance at small and minority business procurement conferences and trade fairs;

e. Records to support internal guidance and encouragement provided to buyers through (1) workshops, seminars, training programs, incentive awards; and (2) monitoring performance to evaluate compliance with the program and requirements; and

f. On a contract-by-contract basis, records to support subcontract award data

including the name, address, and business type and size of each subcontractor. (This item is not required on a *contract – by – contract basis* for company or division-wide commercial plans.)

g. Other records to support your compliance with the subcontracting plan: (Please describe)

_____

_____

8. Timely Payments to Subcontractors

FAR 19.702 requires your company to establish and use procedures to ensure the timely payment of amounts due pursuant to the terms of your subcontracts with small business concerns, 8(a), SDB, women-owned small business, HubZone and service disabled veteran-owned small business concerns.

Your company has established and used such procedures: ___X___ yes _____ no

9. Description of Good Faith Effort

Maximum practicable utilization of small, 8(a), small disadvantaged, woman-owned, HubZone small and service disabled veteran owned concerns as subcontractors in Government contracts is a matter of national interest with both social and economic benefits. **When a contractor fails to make a good faith effort to comply with a subcontracting plan, these objectives are not achieved, and 15 U.S.C. 637(d) (4) (F) directs that liquidated damages shall be paid by the contractor.** In order to demonstrate your compliance with a good faith effort to achieve the small, SDB, WOSB, HubZone and SDVOSB small business subcontracting goals, outline the steps your company plans to take. These steps will be negotiated with the contracting official prior to approval of the plan.

Subcontractor Info

SIGNATURE PAGE

Signatures Required:

This subcontracting plan was submitted by:

Signature: Personnel

Typed Name:

Title: Director, Central Procurement Services

Date: 10/20/2009

This plan was reviewed by:

Signature: Personnel

Typed Name:

Title: Contracting Officer Date: 17 Dec 09

This plan was reviewed by:

Signature: Personnel

Typed Name:

Title: HHS Small Business Specialist (SBS) Date: 12/23/09

This plan was reviewed by:

Signature: PCR RESERVES THE RIGHT TO REVIEW

Typed Name:

Title: Small Business Administration Procurement Center Representative

Date:

EQUITABLE OPPORTUNITY

Subcontractor Info

MISSION AND OBJECTIVES

Subcontractor Info

VENDOR DIVERSITY

Subcontractor Info

Revised January 2008

Subcontractor Info

Revised January 2008

Attachment 5 :
Safety and Health

HHSAR 352.223-70 SAFETY AND HEALTH (JANUARY 2006)

(a) To help ensure the protection of the life and health of all persons, and to help prevent damage to property, the Contractor shall comply with all Federal, State and local laws and regulations applicable to the work being performed under this contract. These laws are implemented and/or enforced by the Environmental Protection Agency, Occupational Safety and Health Administration and other agencies at the Federal, State and local levels (Federal, State and local regulatory/enforcement agencies).

1. In addition, the following regulations must be followed when developing and implementing health and safety operating procedures and practices for both personnel and facilities involving the use or handling of hazardous materials and the conduct of research, development, or test projects:

   (1) 29 CFR 1910.1030, Bloodborne pathogens; 29 CFR 1910.1450, Occupational exposure to hazardous chemicals in laboratories; and other applicable occupational health and safety standards issued by the Occupational Health and Safety Administration (OSHA) and included in 29 CFR Part 1910. These regulations are available at: http://www.osha.gov/comp-links.html

   (2) Nuclear Regulatory Commission Standards and Regulations, pursuant to the Energy Reorganization Act of 1974 (42 U.S.C. 5801 et seq.). Copies may be obtained from the U.S. Nuclear Regulatory Commission, Washington, DC 20555-0001.

2. The following guidelines are recommended for use in developing and implementing health and safety operating procedures and practices for both personnel and facilities:

   (1) Biosafety in Microbiological and Biomedical Laboratories, CDC and NIH, HHS. This publication is available at http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm

   (2) Prudent Practices for Safety in Laboratories (1995), National Research Council, National Academy Press, 500 Fifth Street, NW., Lockbox 285, Washington, DC 20055 (ISBN 0-309-05229-7). This publication can be obtained by telephoning 800-624-8373. It also is available at http://www.nap.edu/catalog/4911.html.

(b) Further, the Contractor shall take or cause to be taken additional safety measures as the Contracting Officer, in conjunction with the project or other appropriate officers, determines to be reasonably necessary. If compliance with these additional safety measures results in an increase or decrease in the cost or time required for performance of any part of work under this contract, an equitable adjustment will be made in accordance with the applicable "Changes" clause set forth in this contract.

(c) The Contractor shall maintain an accurate record of, and promptly report to the Contracting Officer, all accidents or incidents resulting in the exposure of persons to toxic substances, hazardous materials or hazardous operations; the injury or death of any person; and/or damage to property incidental to work performed under the contract and all violations for which the Contractor has been cited by any Federal, State or local regulatory/enforcement agency. The report shall include a copy of the notice of violation and the findings of any inquiry or inspection, and an analysis addressing the impact these violations may have on the work remaining to be performed. The report shall also state the required action(s), if any, to be taken to correct any violation(s) noted by the Federal, State or local regulatory/enforcement agency and the time frame allowed by the agency to accomplish the necessary corrective action.

(d) If the Contractor fails or refuses to comply with the Federal, State or local regulatory/enforcement agency's directive(s) regarding any violation(s) and prescribed corrective action(s), the Contracting Officer may issue an order stopping all or part of the work until satisfactory corrective action (as approved by the Federal, State or local regulatory/enforcement agencies) has been taken and documented to the Contracting Officer. No part of the time lost due to any stop work order shall be subject to a claim for extension of time or costs or damages by the Contractor.

(e) The Contractor shall insert the substance of this clause in each subcontract involving toxic substances, hazardous materials, or hazardous operations. Compliance with the provisions of this clause by subcontractors will be the responsibility of the Contractor.

(End of Clause)

Attachment 6 :
Procurement of Certain Equipment

PROCUREMENT OF CERTAIN EQUIPMENT. NIH(RC)-7

Notwithstanding any other clause in this contract, the Contractor will not be reimbursed for the purchase, lease, or rental of any item of equipment listed in the following Federal Supply Groups, regardless of the dollar value, without the prior written approval of the Contracting Officer.

67 - Photographic Equipment
69 - Training Aids and Devices
70 - General Purpose ADP Equipment, Software, Supplies and Support (Excluding 7045-ADP Supplies and Support Equipment.)
71 - Furniture
72 - Household and Commercial Furnishings and Appliances
74 - Office Machines and Visible Record Equipment
77 - Musical Instruments, Phonographs, and Home-type Radios
78 - Recreational and Athletic Equipment

When equipment in these Federal Supply Groups is requested by the Contractor and determined essential by the Contracting Officer, the Government will endeavor to fulfill the requirement with equipment available from its excess personal property sources, provided the request is made under a cost-reimbursement contract. Extensions or renewals of approved existing leases or rentals for equipment in these Federal Supply Groups are excluded from the provisions of this article.

Attachment 7 :
Research Patient Care Costs

RESEARCH PATIENT CARE COSTS -- NIH(RC)-11

(a) Research patient care costs are the costs of routine and ancillary services provided to patients participating in research programs described in this contract.

(b) Patient care costs shall be computed in a manner consistent with the principles and procedures used by the Medicare Program for determining the part of Medicare reimbursement based on reasonable costs. The Diagnostic Related Group (DRG) prospective reimbursement method used to determine the remaining portion of Medicare reimbursement shall not be used to determine patient care costs. Patient care rates or amounts shall be established by the Secretary of HHS or his duly authorized representative.

(c) Prior to submitting an invoice for patient care costs under this contract, the contractor must make every reasonable effort to obtain third party payment, where third party payors (including Government agencies) are authorized or are under a legal obligation to pay all or a portion of the charges incurred under this contract for patient care.

(d) The contractor must maintain adequate procedures to identify those research patients participating in this contract who are eligible for third party reimbursement.

(e) Only those charges not recoverable from third party payors or patients and which are consistent with the terms and conditions of the contract are chargeable to this contract.

Attachment 8 :
Disclosure of Lobbying Activities, SF-LLL

DISCLOSURE OF LOBBYING ACTIVITIES

Approved by OMB
0348-0046

Complete this form to disclose lobbying activities pursuant to 31 U.S.C. 1352
(See reverse for public burden disclosure.)

1. Type of Federal Action:	2. Status of Federal Action:	3. Report Type:
☐ a. contract b. grant c. cooperative agreement d. loan e. loan guarantee f. loan insurance	☐ a. bid/offer/application b. initial award c. post-award	☐ a. initial filing b. material change **For Material Change Only:** year _____ quarter _____ date of last report _____

4. Name and Address of Reporting Entity:	5. If Reporting Entity in No. 4 is a Subawardee, Enter Name and Address of Prime:
☐ Prime ☐ Subawardee Tier _____, *if known*: **Congressional District**, *if known*: 4c	**Congressional District**, *if known*:
6. Federal Department/Agency:	**7. Federal Program Name/Description:** CFDA Number, *if applicable*: _____
8. Federal Action Number, *if known*:	**9. Award Amount**, *if known*: $
10. a. Name and Address of Lobbying Registrant (*if individual, last name, first name, MI*):	**b. Individuals Performing Services** (*including address if different from No. 10a*) (*last name, first name, MI*):
11. Information requested through this form is authorized by title 31 U.S.C. section 1352. This disclosure of lobbying activities is a material representation of fact upon which reliance was placed by the tier above when this transaction was made or entered into. This disclosure is required pursuant to 31 U.S.C. 1352. This information will be available for public inspection. Any person who fails to file the required disclosure shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.	Signature: _____ Print Name: _____ Title: _____ Telephone No.: _____ Date: _____
Federal Use Only:	Authorized for Local Reproduction Standard Form LLL (Rev. 7-97)

INSTRUCTIONS FOR COMPLETION OF SF-LLL, DISCLOSURE OF LOBBYING ACTIVITIES

This disclosure form shall be completed by the reporting entity, whether subawardee or prime Federal recipient, at the initiation or receipt of a covered Federal action, or a material change to a previous filing, pursuant to title 31 U.S.C. section 1352. The filing of a form is required for each payment or agreement to make payment to any lobbying entity for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with a covered Federal action. Complete all items that apply for both the initial filing and material change report. Refer to the implementing guidance published by the Office of Management and Budget for additional information.

1. Identify the type of covered Federal action for which lobbying activity is and/or has been secured to influence the outcome of a covered Federal action.

2. Identify the status of the covered Federal action.

3. Identify the appropriate classification of this report. If this is a followup report caused by a material change to the information previously reported, enter the year and quarter in which the change occurred. Enter the date of the last previously submitted report by this reporting entity for this covered Federal action.

4. Enter the full name, address, city, State and zip code of the reporting entity. Include Congressional District, if known. Check the appropriate classification of the reporting entity that designates if it is, or expects to be, a prime or subaward recipient. Identify the tier of the subawardee, e.g., the first subawardee of the prime is the 1st tier. Subawards include but are not limited to subcontracts, subgrants and contract awards under grants.

5. If the organization filing the report in item 4 checks "Subawardee," then enter the full name, address, city, State and zip code of the prime Federal recipient. Include Congressional District, if known.

6. Enter the name of the Federal agency making the award or loan commitment. Include at least one organizational level below agency name, if known. For example, Department of Transportation, United States Coast Guard.

7. Enter the Federal program name or description for the covered Federal action (item 1). If known, enter the full Catalog of Federal Domestic Assistance (CFDA) number for grants, cooperative agreements, loans, and loan commitments.

8. Enter the most appropriate Federal identifying number available for the Federal action identified in item 1 (e.g., Request for Proposal (RFP) number; Invitation for Bid (IFB) number; grant announcement number; the contract, grant, or loan award number; the application/proposal control number assigned by the Federal agency). Include prefixes, e.g., "RFP-DE-90-001."

9. For a covered Federal action where there has been an award or loan commitment by the Federal agency, enter the Federal amount of the award/loan commitment for the prime entity identified in item 4 or 5.

10. (a) Enter the full name, address, city, State and zip code of the lobbying registrant under the Lobbying Disclosure Act of 1995 engaged by the reporting entity identified in item 4 to influence the covered Federal action.

    (b) Enter the full names of the individual(s) performing services, and include full address if different from 10 (a). Enter Last Name, First Name, and Middle Initial (MI).

11. The certifying official shall sign and date the form, print his/her name, title, and telephone number.

Attachment 9 :
Government Property - Schedule

REPORT OF GOVERNMENT OWNED, CONTRACTOR HELD PROPERTY

CONTRACTOR:	CONTRACT NUMBER
ADDRESS	REPORT DATE:
	FISCAL YEAR:

CLASSIFICATION	BEGINNING OF PERIOD		ADJUSTMENTS			END OF PERIOD	
	#ITEMS	VALUE	GFP ADDED	CAP ADDED	DELETIONS	#ITEMS	VALUE
LAND>=$25K							
LAND<$25K							
OTHER REAL>=$25K							
OTHER REAL<$25K							
PROPERTY UNDER CONST>=$25K							
PROPERTY UNDER CONST<$25K							
PLANT EQUIP>=$25K							
PLANT EQUIP<$25K							
SPECIAL TOOLING >= $25K							
SPECIAL TOOLING<$25K							
SPECIAL TEST EQUIP>=$25K							
SPECIAL TEST EQUIP<$25K							
AGENCY PECULIAR>=$25K							
AGENCY PECULIAR<$25K							
MATERIAL>=$25K (CUMULATIVE)							
PROPERTY UNDER MFR>=$25K							
PROPERTY UNDER MFR<$25K							

SIGNED BY:

(SIGNATURE) _____

(NAME PRINTED) _____

(TITLE) _____

DATE SIGNED:

_____

(TELEPHONE) _____

Attachment 10 :
Commitment To Protect Non-Public Information

Commitment to Protect Non-Public Information – Contractor Agreement

Access to sensitive information from the files of the National Institutes of Health (NIH) is required in the performance of my official duties, under contract number _____ between (NIH I/C Name or Component) _____ and my employer (Employer's Name) _____. I agree that I shall not release, publish, or disclose such information to unauthorized personnel, and I shall protect such information in accordance with relevant laws and regulations available for research and review at any Law Library. Among these laws may be various provisions of:

a) 18 U.S.C. 641 (Criminal Code: Public Money, Property or Records; 2pgs.long)

b) 18 U.S.C. 1905 (Criminal Code: Disclosure of Confidential Information; 2 pgs. long)

c) Public Law 96-511 (Paperwork Reduction Act; Encyclopedic in length)

I affirm that I have received a written and/or verbal briefing by my company concerning my responsibilities under this agreement. I understand that violation of this agreement may subject me to criminal and civil penalties.

Signed: _____
Type or Print Name: _____
Date: _____
Witnessed by: _____
Date: _____

Copies are to be retained by: NIH Project Officer
Contractor's Contract Management
Individual Signatory

Attachment 11 :
Roster of Employees Requiring Suitability Investigations

ROSTER OF EMPLOYEES REQUIRING SUITABILITY INVESTIGATIONS

CAN:
RFP/Contract #:
Contract Title:
PO:
CO:

Contractor:
Contractor Contact:
Contractor E-mail:

TO BE FILLED IN BY CONTRACTOR:							TO BE FILLED OUT BY NCI PROJECT OFFICER and ISSO:				TO BE FILLED OUT BY CONTRACTOR:							
							Suitability Designation	Background Investigation		Questionaire Required					Existing Clearance or Application			
Last Name	First Name	e-mail	Position	Data Access	NDA Submitted	Sec Awareness Training Completed	1C, 5C, 6C	NACI, NACIC, MBI, LBI, BI	Estimated Cost	SF85 or SF85P	Questionaire Submittted	HHS Credit Release Submittted	OF 306 Submittted	Fingerprinting Completed	Agency	Clearance Level	Date	Comment

Attachment 12 :
Employee Separation Checklist

EMPLOYEE SEPARATION CHECKLIST

Contractor: _____ Contract No: _____

Departing Staff Member's Name: _____ Separation Date: _____

Check and complete one of the columns below as appropriate:

I. FRIENDLY SEPARATION		II. UNFRIENDLY SEPARATION	
Date *(Mandatory)*	Action	Date *(Mandatory)*	Action
	Remove all network and system access privileges.		Disable system access as quickly as possible–preferably just before the individual is notified of his or her dismissal.
	Collect any authentication tokens.		Terminate access to systems immediately when an employee notifies the Department of a resignation that is on unfriendly terms.
	Retrieve any access cards or Departmental identification badges.		Notify support functions (e.g., help desk) that an employee is no longer authorized access.
	Recover all keys.		Restrict the area and function of employees during the period between termination and leaving.
	Brief employee on continuing confidentiality and privacy responsibilities.		Immediately notify the Project Officer, appropriate NIH security officials, and the assigned IT Systems Manager of the time of removal.
	Review any employee contracts that remain valid after separation.		Request the Project Officer to have the combinations changed on all locks to which the contractor employee has access.
	Return property belonging to the United States Government.		Collect any authentication tokens.
	Identify any unique problems, filing schemes, or data backups created by the employee.		Retrieve any access cards or Departmental identification badges.
	Instruct employees on proper "clean up" procedures for their personal computers (PC) before leaving.		Recover all keys.
	Determine the employee's access termination date, and notify the Project Officer, appropriate NIH security officials, and the assigned IT Systems Manager within 24 hours of the time of termination.		Review the employee's duties and responsibilities under this contract with the Project Officer and assess the level of risk to the Government.
	Notify the Project Officer in writing upon completion of these actions.		Escort individual off premises in cases where the potential for retaliation is high.
			Notify the Project Officer in writing upon completion of these actions.

CERTIFICATION: By signing below, I certify that the above actions were taken on the dates indicated.

_____ _____

Signature and Date Typed Name of Individual Authorized to Certify for Contractor

_____

Title of Individual Authorized to Certify for Contractor

Attachment 13 :
Deliverables

Item	Description	Quantity	Delivery Schedule
1	Quarterly Technical Progress Reports	1 electronic copy to COTR and CO 1 hardcopy to COTR 1 original hardcopy to CO	The initial report will be submitted for the first full three months of the contract performance including any fractional part of the initial month. Thereafter, the reporting period shall consist of three full calendar months. Reports are due on or before the 15th calendar day of the month, following the end of the quarterly reporting period.
2	Semi-Annual Technical Progress Reports	1 electronic copy to COTR and CO 1 hardcopy to COTR 1 original hardcopy to CO	The initial report will be submitted for the first full six months of the contract performance including any fractional part of the initial month. Thereafter, the reporting period shall consist of six full calendar months. Quarterly reports will not be submitted the month the semi-annual report is due. Reports are due on or before the 15th calendar day of the month, following the end of the semi-annual reporting period.
3	Annual Technical Progress Reports	1 electronic copy to COTR and CO 1 hardcopy to COTR 1 original hardcopy to CO	An annual report will not be required for the period when the Final Report is due. A Semi-Annual Report shall not be submitted when an Annual Report is due. Reports are due on or before the 15th calendar day of the month, following the end of the reporting period.
4	Annual Technical Progress Report for Clinical Research Study Populations - Only for those contracts involving human subjects.	1 electronic copy to COTR and CO 1 hardcopy to COTR 1 original hardcopy to CO	An annual report will not be required for the period when the Final Report is due. A Semi-Annual Report shall not be submitted when an Annual Report is due. Reports are due on or before the 15th

			calendar day of the month, following the end of the reporting period.
5	Draft Final Technical Report	1 electronic copy to COTR and CO 1 hardcopy to COTR 2 original hardcopies to CO	The report is due 60 calendar days prior to the completion date of this contract. The COTR will review the draft report and provide the Contracting Officer with comments within 20 calendar days after receipt. The Final Report shall be corrected by the Contractor, if necessary, and the final version delivered as specified in the above paragraph.
6	Final Technical Report	1 electronic copy to COTR and CO 1 hardcopy to COTR 1 original hardcopy to CO	The Contractor shall submit, with the Final Report, a summary (not to exceed 200 words) of salient results achieved during the performance of the contract. The report is due 30 days before the completion date of the contract.
7	Quarterly Teleconferences along with Agenda and Minutes	1 electronic copy to COTR	See "Quarterly Teleconferences with the COTR" section below for details.

"Quarterly Teleconferences with the COTR"

The Contractor shall hold teleconferences 4 times per year (once every three months) with the COTR. Additional Contractor staff and the Contracting Officer shall participate as needed. The exact meeting schedule will be established by the Contractor and COTR within fifteen (15) working days of contract award. The main purpose of these meetings is to apprise the COTR of technical achievements or hurdles related to the program goals and objectives. Additional topics may include budgetary issues, subcontracting agreements, travel requirements, planning site visits; and will be determined by the Contractor, with input from the COTR, prior to the meeting. The Contractor shall arrange the teleconferences, in consultation with the COTR.

A. Ten (10) working days prior to each teleconference, the Contractor shall submit to the COTR, for review, an electronic version of the proposed meeting agenda, including progress on action items from the previous teleconference. Upon approval of the COTR, the Contractor shall distribute the final agenda to all of the teleconference participants. The COTR shall provide feedback in a timely manner, and the Contractor shall send out the final agenda, call-in information, and updated minutes from the prior teleconference to all participants at least two (2) working days prior to the teleconference.

B. Within five (5) working days following each teleconference, the Contractor shall submit a summary of the meeting discussions and list of action items, including any proposed due dates for the actions, to the COTR for review. The COTR shall submit any comments or approval for distribution, to the Contractor within five (5) working days of receipt of the summary of the meeting discussions. The Contractor shall distribute the meeting minutes and action items to all the meeting participants within two (2) working days of receiving the COTR comments.

b. Other Reports and Deliverables (Delivery Schedule)

Item	Description	Quantity	Delivery Schedule
1	Source Code and Object Code	1 electronic copy to COTR	The Contractor also shall deliver to the Government, upon expiration date of the contract, all source code and object code developed, modified, and/or enhanced under this contract.
2	Epitope Data	(see "delivery schedule" to the right)	Immune epitope information shall be submitted to the IEDB (www.ImmuneEpitope.org) within six (6) months of validation, or as determined by the COTR, in consultation with the Contracting Officer and Contractor. Reference SECTION C, under "Other Reports/Deliverables", of this contract for additional information.
3	Prediction Tools	(see "delivery schedule" to the right)	Contract generated software shall be made available, within six (6) months of validation, through publicly accessibly web and databases sites, with first preference given to the IEDB (www.ImmuneEpitope.org). If the IEDB does not choose to host the tool, it shall be made accessible through other public websites or repositories identified

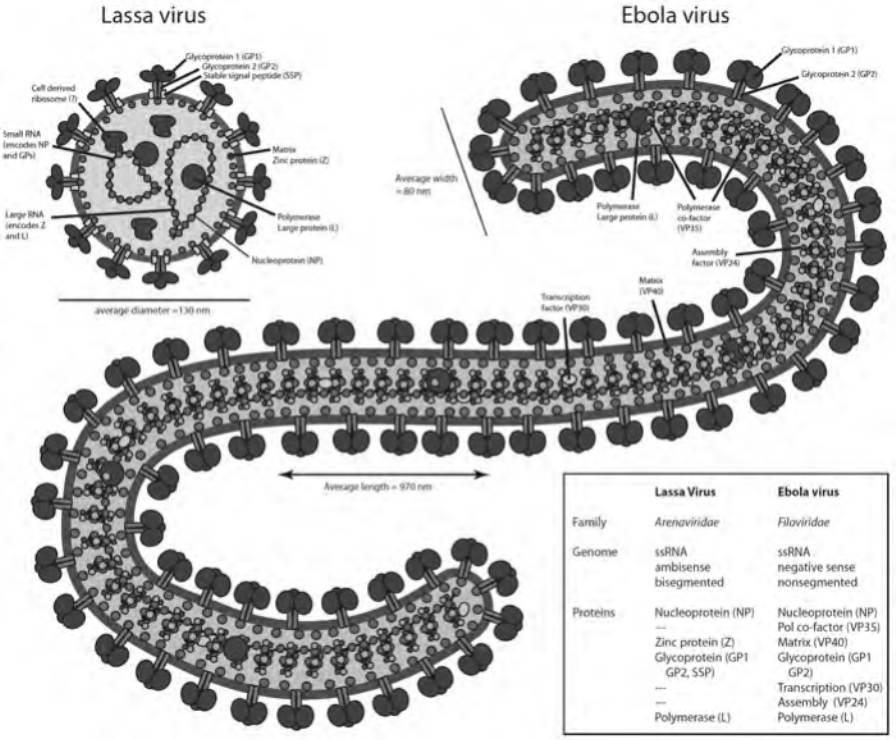
			by the Contractor in consultation with the COTR. Reference SECTION C, under "Other Reports/Deliverables", of this contract for additional information.
4	Intellectual Property	(see "delivery schedule" to the right)	The Contractor is required to report to the U.S. Government all inventions made in the performance of the project, as specified at FAR 52.227-11 (Bayh-Dole Act). Reference SECTION C, under "Other Reports/Deliverables", of this contract for additional information.

Addressee	Deliverable Item No	Quantity
Contracting Officer's Technical Representative (COTR): Timothy A. Gondré-Lewis, Ph.D. Program Officer Immunoregulation Section, Basic Immunology Branch Division of Allergy, Immunology, and Transplantation National Institute of Allergy and Infectious Diseases, NIH, HHS 6610 Rockledge Drive, Room 3011 Bethesda, MD 20892-6601 (20817 for Express Delivery)	As stated in the table(s) above.	As stated in the table(s) above.
Contracting Officer (CO): Donald E. Collie Contracting Officer/Team Leader Allergy, Immunology, and Transplantation Research Contract Branch (AIT-RCB) Office of Acquisitions (OA) DEA/NIAID/NIH/DHHS 6700B Rockledge Drive, Room 3109, MSC 7612	As stated in the table(s) above.	As stated in the table(s) above.

Bethesda, MD 20892-7612 For express mail: 6700-B Rockledge Drive, Room 3214 Bethesda, MD 20817		

SCIENTIFIC UPDATE
FINAL SUMMARY
(includes Quarters 19 and 20 SUMMARY for 4/1/14 – 9/30/14)

Roles of protective or pathogenic B cell epitopes in human Lassa fever



	Lassa Virus	Ebola virus
Family	*Arenaviridae*	*Filoviridae*
Genome	ssRNA ambisense bisegmented	ssRNA negative sense nonsegmented
Proteins	Nucleoprotein (NP) --- Zinc protein (Z) Glycoprotein (GP1 GP2, SSP) --- --- Polymerase (L)	Nucleoprotein (NP) Pol co-factor (VP35) Matrix (VP40) Glycoprotein (GP1 GP2) Transcription (VP30) Assembly (VP24) Polymerase (L)

PI: James E. Robinson, M.D.
Tulane University
1430 Tulane Avenue
New Orleans, Louisiana 70112
DUNS No.: 053785812

Contract HHSN272200900049C under BAA-NIAID-DAIT-NIHAI2008031
" B Cell Epitope Discovery and Mechanisms of Antibody Protection**"**

August 7, 2014

OVERVIEW OF OVERALL PROGRESS

MILESTONE 1: Derive human monoclonal antibodies (huMAbs) to Lassa virus (LASV) proteins. We have cloned and expressed over 120 huMabs to LASV glycoproteins (GPs) and 10 huMAbs to the LASV NP. huMAbs have also been derived from a Nigerian survivor of lineage II LASV, including 5 neutralizing antibodies.

MILESTONE 2: Map linear or conformational B cell epitopes using mutant and wild-type recombinant LASV proteins, and overlapping synthetic peptides. We completed a comprehensive epitope map of Lassa viruses of lineage IV circulating in Sierra Leone. We have identified three competition groups of LASV GP1 huMAbs and mapped two GP1 epitopes. We have identified five competition groups of LASV GP2 huMAbs and mapped six GP2 epitopes. Competition groups can represent more than one epitope. Most GP epitopes are conformational, but we have identified 3 linear epitopes (all in GP2) to date. Cross-reactivities in B cell epitopes between Old/New World arenaviruses have also been established. We have measured huMAb binding to LASV proteins by Biocore.

Milestone 3: Determine X-ray crystallographic structures of the GP, NP and Z proteins of LASV and other arenaviruses in complex with human MAbs. We have determined the crystal structure of oligomeric lymphocytic choriomeningitis virus (LCMV) LCMV GP, which illustrates oligomeric association of a prefusion Old World arenavirus GP and provides template for mapping the LASV antibody epitopes. We have determined that the GP trimer assembly is mediated by three sites of GP-GP interaction: (a) The N terminus of GP1 forming hydrophobic interactions to the neighboring monomer's fusion loop, (b) The C terminus of GP2 to the base of the neighboring monomer's fusion loop, and (c) a disordered loop of GP2 that extends into the center of the trimer axis. We determined the crystal structure of LASV NP N-terminal domain, which revealed the RNA binding site and RNA gate. We determined the crystal structure of the LASV NP C-terminal domain, which revealed the 3'5'-exonuclease fold and immunosuppressive function in digestion of dsRNA. The crystal structures of NP have allowed us to epitope map several human and murine MAbs. We have also determined the crystal structure of the LASV Z oligomer.

Milestone 4. Develop novel screening methods for LASV B cell epitope identification, including deuterium exchange mass spectrometry (DXMS) and small angle X-ray scattering. We have developed DXMS as a tool to identify linear and conformational epitopes. DXMS is now widely used by other B cell epitope groups.

Milestone 5: Define the kinetics of IgM- and IgG-responses to defined epitopes on GP, NP and Z proteins of LASV using variations of LF ELISA and ELISPOT assays in acutely-infected, convalescent, and previously-exposed persons. We have determined kinetics of IgM- and IgG-responses in individuals infected with LASV, and identified inhibition of antibody class switching as a potential pathogenic mechanism. We will continue to assess immunodominance patterns to specific B epitopes and to define responses to specific epitopes in LF patients as a high priority.

Milestone 6: Quantify and characterize anti-LASV neutralizing and enhancing antibody responses in a well-characterized cohort of persons exposed to diverse strains of LASV at different stages and with different severities of illness. We have shown that only about half of LF survivors develop neutralizing antibodies. We have also expressed 16 huMAbs, which

have neutralizing activity, representing four epitope groups (GP1-A and GP2-A1, -A2 and C). We are actively characterizing the mechanism of neutralization by huMAbs. No evidence for enhancement has been obtained.

Milestone 7: Validate protective or pathogenic role of antibodies to defined epitopes on GP, NP and Z proteins of LASV using *in vivo* challenge and protection studies in our well-established guinea pig and non-human primate models of LF. We have achieved 100% protection from lethal challenge with Lassa virus in guinea pigs and *Cynomolgus macaques* by injection of single Lassa virus human monoclonal antibodies. These immunotherapeutics are similar to ZMapp, a triple monoclonal antibody cocktail therapy for Ebola virus, but are more potent even as single monoclonal antibodies. We have also identified several partially protective huMANS in guinea pig and macaques. In one case a completely protective antibody appears to have "evolved" from a partially protective antibody by somatic mutation. Lessons learned with Lassa virus immunotherapeutics can be used to improve Ebola virus immunotherapeutics. We have also developed new methods for large scale production of huMAbs in serum-free GMP compliant cell lines.

Milestone 8: Determine LASV sequence diversity and impact on recognition of B cell epitopes by LF patients (Harvard/Broad Institute). We have generated the largest catalog of deep sequencing data for a viral hemorrhagic fever agent. This and other data suggest that LASV strains likely spread out of Nigeria ~ 500 years ago, but only made it into Sierra Leone around 150 years ago. We have also shown that intrahost single nucleotide variants (iSNVs) predominantly occur in putative LASV GP B cell epitopes indicating selection by the humoral immune system.

Milestone 9: Evaluate all B cell epitopes discovered (under Requirement a) for functionality by comparing these acquired responses with LF clinical disease outcomes to identify the mechanisms of protection or pathogenesis in humans. We enrolled 200-300 cases of confirmed LF per year; 50-75 per Quarter at our clinical sites at Kenema Govdernment Hospital in Sierra Leone and at Irrua Specialist Teaching Hospital (ISTH) in Nigeria. We have correlated IgM and IgG titers to clinical outcomes in all consenting human subjects presenting to Kenema Government Hospital during the project period. We are analyzing a similar dataset collected at ISTH. Epitope-specific ELISA have been produced and we are in the process of analyzing reactivity to specific B cell epitopes and comparing these to clinical outcomes.

Milestone 10: As specified in the RFP, submit information on LASV B cell epitopes to the Immune Epitope Database and Analysis Resource. We have submitted 19 LASV B cell epitopes to IEDB. 100 epitopes will be submitted at the conclusion of the contract.

OVERVIEW OF PROGRESS Q19-20

- We have identified several huMAbs that provide 100% protection from Lassa virus infection in a guinea pig model, as well several that provide only partial (20-40%) protection. Antibodies have been isolated from the same individual. In one case a completely protective antibody appears to have "evolved" from a partially protective antibody by somatic mutation. The three most promising huMAbs in guinea pigs were tested in Cynomolgus macaques where they demonstrated 100% protection.

- Structural studies of arenavirus GP continues. These studies are revealing the structural basis for arenavirus GP assembly and α-dystroglycan receptor binding.

- We have generated the largest catalog of deep sequencing data for a viral hemorrhagic fever agent, with now over 300 Lassa virus strains sequenced. Recently, this capacity has allowed us to rapidly sequence 99 new Ebola virus genomes from Sierra Leone (94% of isolates from the first 24 days of the outbreak), and provided important information about the ongoing outbreak in West Africa. Diseases caused by both Lassa virus and Ebola virus (cover) are now present in West Africa.

BACKGROUND

Because of its high case fatality rate, ability to spread easily by human-human contact, and potential for aerosol release, Lassa virus (LASV), the causative agent of Lassa fever (LF), is classified as a Biosafety Level 4 (BSL-4) and NIAID Biodefense category A agent. The potential use of LASV as a biological weapon directed against civilian or military targets necessitates development of counterthreat measures, such as diagnostic assays, vaccines and therapeutics. The impact of LF in endemic areas of West Africa is immense, and therefore means to diagnose, treat or prevent this viral hemorrhagic fever (VHF) will provide a very significant public health benefit. Our team has produced LASV enzyme-linked immunosorbent assays (ELISA) that are based on recombinant proteins rather than on reagents that must be produced in high containment laboratories. We have also established research programs in Sierra Leone and Nigeria that provide unique clinical and laboratory resources for VHF research. The goals of this program are to use these laboratory and clinical resources to identify novel B cell epitopes on LASV proteins and elucidate mechanisms of antibody-mediated protection or pathogenesis in humans infected with LASV.

A. EPITOPE IDENTIFICATION

MILESTONE 1: Derive human monoclonal antibodies (huMAbs) to LASV proteins (Tulane).

Goal:

- Identify 15-20 Lassa virus GP and 15-20 NP and 10-15 Z MAbs per Quarter.

OVERVIEW OF CONTRACT DELIVERABLES ACHIEVED IN MILESTONE 1.
We exceeded Milestone 1 contract goals.

Technological advances allowed us to greatly exceed **identification** of the relatively modest numbers of huMabs we planned in our program goals. Our original plan was to utilize established, but labor intensive, techniques involving EBV cloning of human B cells to identify (screen) for huMabs recognizing LASV GP, NP and Z. Through modified VH and VL gene cloning methods it is now possible to identify large numbers (hundreds) of huMAbs to LASV proteins in a single screening. While hundreds of huMabs to LASV NP and Z were identified in the first year of the contract greatly exceeding contract goals, as advised by the Epitope Discovery Advisory board we have since focused on generating new human MAbs recognizing LASV GPs.

The new techniques we utilize also allowed for streamlined production and increased yield of purified huMAbs (post-identification). Since the last progress report we have expanded our set of cloned and expressed huMAbs to LASV GP to approximately 120, 17 of which have LASV neutralizing activity (Tables 1 and 2). Although we have accumulated data for a larger group of

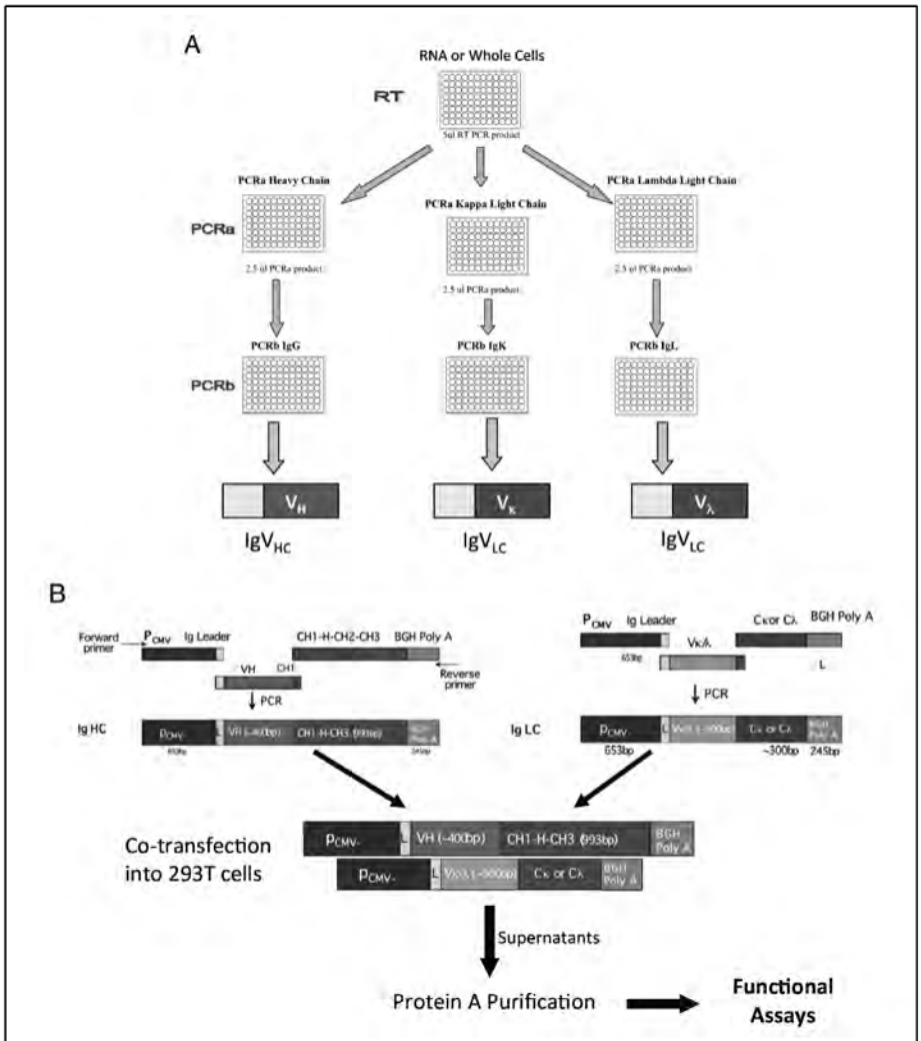


Fig. 1. Molecular Cloning of hMAbs Using Linear Ig Expression Constructs. Immunoglobulin (Ig) VH and VL genes are amplified by reverse transcriptase (RT) and nested PCR (A) and then assembled into functional linear Ig gene expression cassettes (B) composed of three overlapping DNA fragments that form transcriptionally functional full-length Ig heavy and light chain DNA constructs, which can be used directly to co-transfect 293T cells for production of Ig for functional analysis. Figure adapted from [2].

huMAbs, for clarity we focus in this progress report on a representative set of 42 huMAbs to the LASV GPs, with reference to huMabs to LASV NP.

Identify 15-20 Lassa virus GP and 15-20 NP and 10-15 Z MAbs per Quarter. huMAbs provide powerful tools to understand the humoral response to pathogens and to directly exploit the technology for medical benefit via generation of improved diagnostics, immunotherapeutics and vaccines. While antibodies to other LASV proteins, NP and Z are important in the diagnosis of LF, the humoral responses to the surface GPs are of paramount importance in disease outcome and protection from pathogenesis. To identify epitopes that elicit B cell responses in survivors of LASV infection, from the thousands of LASV GP huMabs we identified in intial screening we have produced ~ 120 huMAbs at recognizing LASV GPs in quantities necessary for further characterization. The sources of B cells were peripheral blood mononuclear cells (PBMC) isolated from the blood of LF survivors in Sierra Leone infected with LASV Lineage IV and Nigerian survivors of lineage II LASV at Irrua Specialist Teaching hospital in Nigeria (Figure 1). PBMC were cryopreserved at the KGH or ISTH Lassa Laboratories in Sierra Leone and Nigeria and transported to Tulane in liquid nitrogen dry shippers. We used two methods for transient stimulation of memory B cells. In the first, B cell enriched fractions were cultured on irradiated mature human macrophages in medium supplemented with R848 and IL-2 [4]. Recently, we adapted a more efficient system in which PBMC enriched for memory B cell fractions are cultured at nearly clonal cell densities in

multiple 96 well plates seeded with MS40L feeder cells derived from a murine mesenchymal stem cell line (MS5) engineered to express human CD40L. [5] These cells support robust B cell growth in Iscove's MDM containing 10%FCS, CpG 2006, IL-2, and IL-21. Culture fluids are screened by ELISA for reactivity with purified LASV GPC. RNA is isolated from B cells producing specific IgG to LASV proteins. Heavy and light chain variable region genes are amplified by RT-PCR and inserted into human light (LC) and heavy (HC) chain expression vectors [2]. (Fig. 1). HEK-293T cells are then co-transfected with matched LC and HC constructs to assess expression of individual LASV huMAbs. VH and VL genes are then cloned into efficient expression plasmids for scale-up production of huMAbs in transiently transfected 293T cells [6, 7]. MAbs are purified by Protein A affinity chromatography.

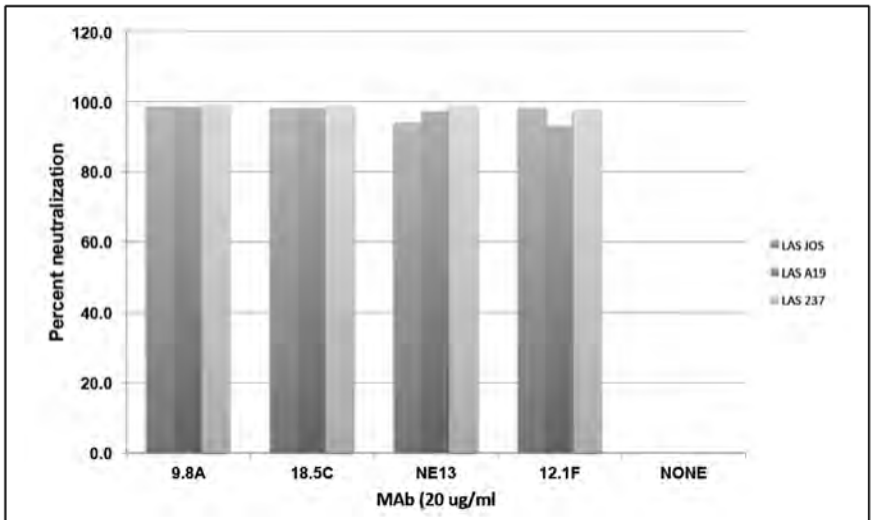


Fig. 2. Neutralization of LASVpp with GPC of various lineages by new Mabs 9.8A, 18.5C, and NE13. 9.8Aand NE13 are from a Nigerian survivor of Lassa fever. 12.1F is included as a positive control. LASV JOS (strain Josiah) is lineage IV, LASV A19 is lineage III, and LASV 237 is lineage II

Progress on epitope mapping and other characterizations of LASV huMabs are described under subsequent Milestones. The antibodies have been placed into groups based on their binding to GP1 or GP2 and cross-competition (Tables 1 and 2). The majority of huMAbs isolated have binding specificity for GP2. One interesting huMab (8.9F) appears to recognize a quaternary epitope. Three cross completion groups are defined for GP1 huMAbs. Likewise, five cross-competition groups have been defined for GP2 huMAbs. It is important to recognize that the cross competition studies provide only rough guidance for epitope mapping. As described under progress of subsequent Milestones, GP1 specific huMAbs of groups GP1-B and C likely represent an overlapping "epitope," and the GP-A cross competition group likely represents two distinct "epitopes," now designed A1 and 2. We also characterized the ability of the LASV huMAbs to bind to overlapping linear peptides a technique often referred to as Pepscan. If the huMAbs failed to bind to any linear peptide, we provisionally classified its epitope as "conformational." Most of the LASV GP huMabs appear to be directed to conformational epitopes. We have now identified two additional linear epitiopes bringing the total to three linear epitopes. We have also assessed the ability of the huMAbs to neutralize LASV. Further details of the ability of huMabs to neutralize LASV and protect in guinea pig and NHP models of LF are described in Milestones 6 and 7. 12 of most recent huMabs were derived from a Nigerian subject. Five (8.11G, 18.5C, NE13, 9.8A, 11.11E) are neutralizing MAbs. Of note two neutralizing MAbs NE13 and 9.8A from the Nigerian subject have a broad neutralizing profile for different LASV lineages (Fig. 2).

Ontogeny of antiLASV GP huMAbs. Sequences from anti-LASV huMAbs revealed that heavy and light chains are derived from diverse sets of human germline lineage genes, without apparent bias between donors toward any given allele, or combination of alleles (Tables 1 and

2). This result suggests that our cloning methods are not selective for any particular antibody lineage. We have also found that most circulating B lymphocyte-derived antibodies show moderate divergence from their presumptive germline genes at the nucleotide level (>90% identity), which is reminiscent of secondary infection with other viruses, such as influenza virus [8-10]. A small subset of LASV huMAbs isolated to date show pronounced divergence from presumptive germline genes, in one or both chains (~65% identity), suggesting an ongoing evolution of the antibody response following the acute LASV infection.

Analysis of 28 independent huMAbs isolated from a single convalescent donor, G355, has generated the best profile to date of a unique and functionally diverse antibody response to the LASV glycoprotein (GPC). Twenty-two of the 28 antibodies do not neutralize LASV (Milestone 6), six antibodies have shown potent neutralization *in vitro*, and one, 37.2D, fully protected guinea pigs in a lethal LASV challenge study (Milestone 7). Five of 6 nAbs from G355 emerged from a common VH germline gene, IGVH-21, with a single exception, 37.2D, which was presumptively derived from IGVH-18. The D gene repertoire is primarily represented by IGHD3-10 and -16 in this donor, with IGHJ4 and J6 representing the J genes. The 37.2D huMAb is unique in the G355 LASV antibody repertoire in that is emerged from IGVH-18, IGHD5-12, and IGHJ6. Future *in vivo* protection studies with the additional nAbs derived from this donor will further establish the diversity of the human antibody repertoire that will generate productive rearrangements with functional neutralization activity against LASV infection. Two similar nAbs, 37.7H and 25.6A, are highly divergent from their common germline VH gene, IGHV3-21, at 66% and 65%, respectively (Table 1, yellow highlights). Interestingly, the large number of mutations in 37.7H and 25.6A resulted in unconventional amino acid residue changes, such as mutation of the cysteine commonly delineating the start of CDR-H3 to a tyrosine. To date the only instances of such significant mutations in the CDR-H3 on anti-LASV GPC-specific antibodies were observed in nAbs 37.7H and 25.6A, and the non-nAb 3.3B. Two other highly related nAbs from G355, 36.9F and 2.9D emerged from the same V, D, and J germline lineages, and display modest mutation rates (91.32% and 93.06% identity to IGVH germline genes, respectively, Table 1, green highlights). Another pair of related antibodies 9.7A and 8.11C was identified in another donor G551 (Table 1, gray highlights). Antibody 3.3B, isolated from a different donor (G502) than 37.7H and 25,6A, is only 65% identical to the germline IGVH3-23 allele, but does not neutralize LASV *in vitro*. The most remarkable feature of IGKV and IGLV germline lineage gene analyses for all current LASV huMAbs was a highly divergent IGKV4 (64%) gene in 36.9F and a moderately divergent IGKV1 (88%) in 10.4B, both nAbs *in vitro* (10.4B partially protected GPs – 40% - in a lethal challenge study – see Milestone 7, Fig. 40). Additionally, the phenylalanine that commonly delineates the end of CDR3-L3 in nAb 10.4B was deleted and/or mutated to a leucine residue.

Overall, a correlation between extremely high divergence from germline lineage genes and neutralization by anti-LASV antibodies has not yet been observed. To date 3 LASV huMAbs that showed partial (10.4B) to complete (19.7E, 37.2D) protection in guinea pigs, and partial protection (19.7E) in NHP are 89 – 92% identical to their presumptive IGVH germline genes. Furthermore, a simple correlation between the length of LASV huMAb CDR-H3 domains and neutralization potential has also not yet been established through these studies; nAb CDR-H3 lengths range from 12 (19.7E, donor G583) to 31 (8.9F, donor LS011) amino acid residues. Only nAb 8.9F has a CDR-H3 > 21 residues, with most other antibodies ranging from 12 to 17 residues. These findings contrast with those observed for many broadly nAbs many HIV gp41 and gp120, which develop only after prolonged period of persistent infection. HIV bNAbs have a range of 14 – 30 residues, but with a majority containing >20 amino acids. The rare emergence of broadly neutralizing antibodies (bnAbs) against HIV-1 only months to years after infection has

been extensively reported. In particular, broadly neutralizing antibodies against HIV-1 rarely emerge in patients [11]. One major contributing factor is the observation that all known HIV-1 bnAbs are highly divergent from germline antibodies, and that germline antibodies cannot bind to the same epitopes as the respective mature antibodies. These observations led to the hypothesis that HIV-1 may have evolved to "use" absent or weakly binding germline-lineage antibodies as an escape mechanism. It has been observed that a high level of somatic mutations is required for bnAbs to accurately target the conserved structures on the HIV-1 Envelopes. Recent studies corroborate that HIV-1 may eliminate strong binding of germline antibodies due to the absence of closer antiviral antibody intermediates as an escape mechanism. Identification of these intermediates – divergent genes from germline alleles – is a focal area of anti-HIV immunotherapeutics research and design of effective vaccine development.

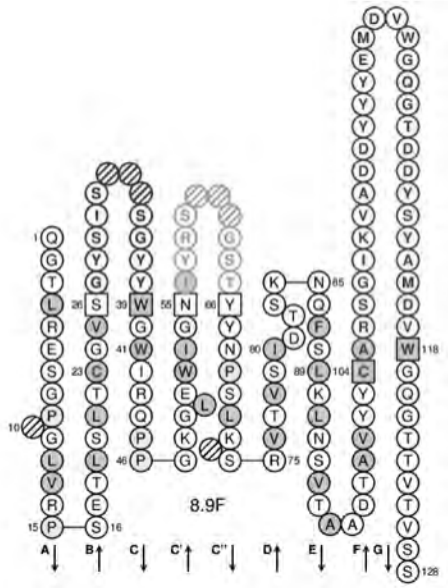


Fig. 3. Sequence of 8.9F an huMAb with a Quaternary epitope. The huMabs has a high unusual 31 residue CDR-H3. This a was derived from a survivor, now working as a nurse in the Lassa ward.

Currently, and despite speculation in the literature regarding long-term sub-clinical antigenemia in LF survivors, it is not known if maturation of the B cell response in convalescent LF is driven by antigen dependent mechanisms. Our results suggest that antibodies to LASV continue to mature over time after resolution of symptoms and discharge from the Lassa Ward. Whether antibody maturation is antigen-independent or is driven by exposure to LASV antigen present in a reservoir or from infected (but perhaps damaged or dead cells) is unknown. All antibodies isolated to date, with the exception of a single nAb, 8.9F, recognize recombinant LASV GPC antigen immobilized on ELISA plates, or soluble antigen in immunoprecipitation assays. The unique feature of nAb 8.9F lies in its ability to neutralize LASV pseudoparticles, despite its inability to bind soluble antigen, thereby prompting the assumption that this antibody recognizes a quaternary epitope in GPC. Current understanding points to a trimerized complex of LASV GP1, GP2, and SSP molecules displayed on the surface of the virion. The 8.9F antibody was derived from donor LS011, emerged from the GHV4-b allele, which also gave rise to the non-neutralizing 36.7D that joined with IGHD6-19, and IGHJ6. The 31 residue CDR-H3 in 8.9F may account for its ability to detect a potentially complex and large quaternary epitope in virion-associated LASV GPC (Fig. 3). This antibody was derived from a LF survivor on the KGH team, who has remained consistently exposed to LASV through work-related activities.

Other antibodies with unusual features include 8.4F and 14.3D, which are both non-neutralizing and contained in the GP-2D complementation group (Table 1, red highlights). Both 8.4F and 14.3D contain unusual CDR-3H sequences with two internal cysteines, potentially constraining a 4 amino acid residue domain (Fig. 4). 6.6C and 37.9D also have this rather unusual potential dicysteine loop in the CDR-H3 region (Fig. 4). Classification of these antibodies is in process to determine if they also fall into complementation group GP2-D. 8.4F, 14.3D, 6.6C and 37.9D were cloned from the memory B cells of four different LF survivors. These types of internal CDR loops constrained by cysteines are common in shark, camel, and cow antibodies, and are often

correlated with high affinity binding to target antigens. This feature has also been observed in natural human antibodies with high affinity for target antigens.

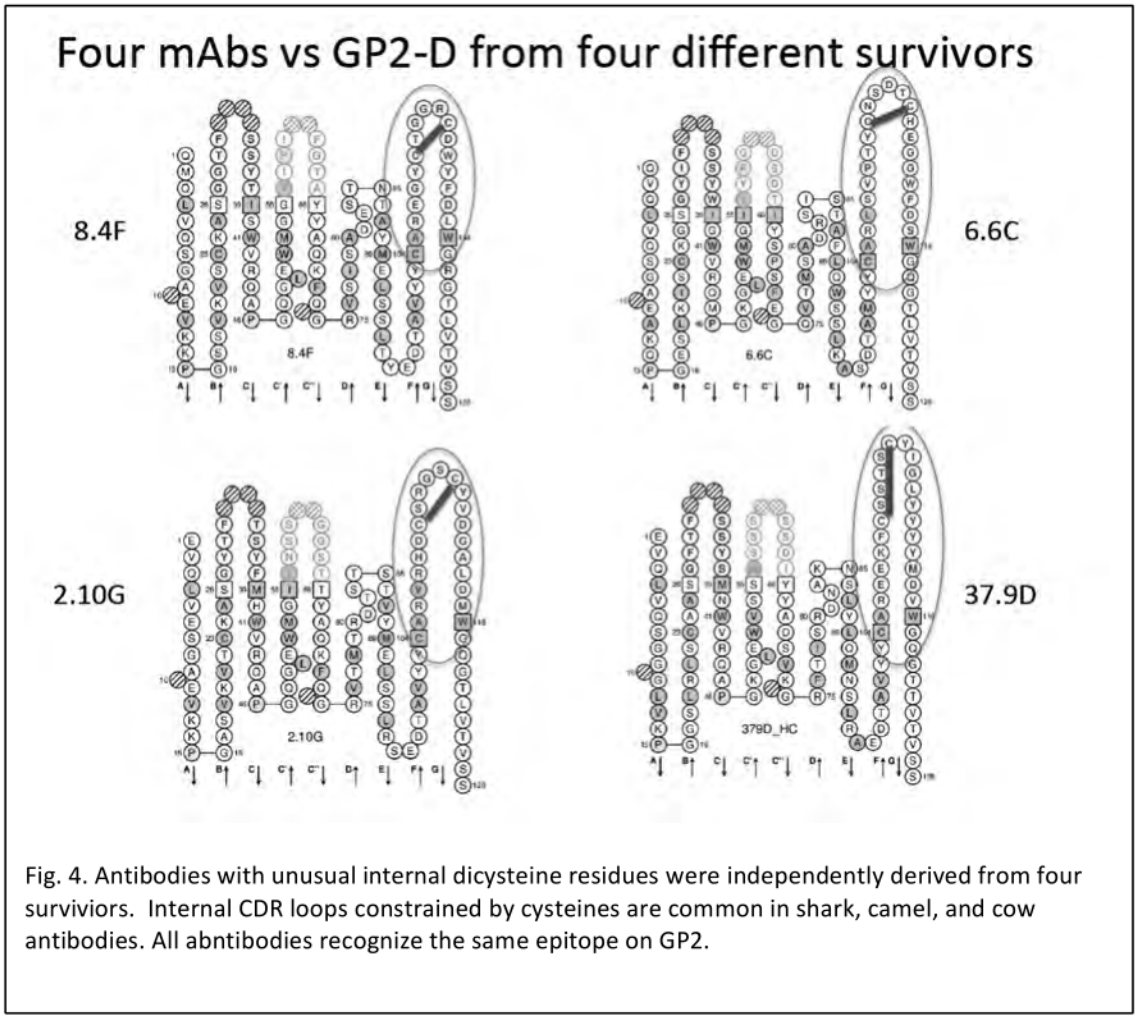


Fig. 4. Antibodies with unusual internal dicysteine residues were independently derived from four surviviors. Internal CDR loops constrained by cysteines are common in shark, camel, and cow antibodies. All abntibodies recognize the same epitope on GP2.

Table 1. Selected LASV Glycoprotein huMAbs isolated from human survivors of Lassa fever in Sierra Leone

Group	huMAb	Neut-tralizing	Epitope type	Epitope (amino acids)	V	D	J		Seq CDR-H3	Mutation	patient
GP1-A	19.7E	+	conformational	111-117	Proprietary Info					92.36	G583
GP1-B	21.6 G	-	conformational	121-133						93.4	G355
GP1-B	2.4F	-	conformational	122-133						96.53	G583
GP1-C	5.11A	-	tbd	tbd						95.14	G583
GP1-C	36.7D	-	tbd	tbd						91.31	G355
GP1-C	19.5A	-	conformational	tbd, GP1 dimer						92.44	G583
GP-1C	21.5F	-	conformational	tbd						90.97	G355
GP1-C	3.3B	-	conformational	126-133						64.93	G502
GP1-C	5.6H	-	conformational	126-133						?	G583
GP1-C	6.7B		tbd	tbd						96.53	G583
GP1-BC	18.5D	-	conformational	121-133						98.28	G617
GP1-BC	39.3G	-	conformational	121-133						95.49	G355
GP1-BC	9.9F	-	tbd	tbd						90.03	G673
GP1-BC	37.4E	-	conformational	121-133						96.22	G355
GP1-?	12.1F	+	tbd	tbd						91.23	G448
GP2-A1	37.2D	+	conformational	270-278						89.24	G355
GP2-A1	25.10C	+	conformational	270-278						91.32	G355
GP2-A1	36.1F	+	conformational	270-278						87.02	G355
GP2-A2	37.7H	+	conformational	364-370; 377-388						66.32	G355
GP2-A2	25.6A	+	conformational	364-370; 377-388						65.26	G355
GP2-A2	36.9F	+	conformational	364-370; 377-388						91.32	G355
GP2-A2	2.9D	+	conformational	364-370; 377-388						93.06	G355
GP2-B	22.5D	-	linear	300-315						95.49	G355
GP2-B	4.1F	-	linear	303-309						92.71	G355
GP2-B	8.8B	-	linear	303-309						94.44	G400
GP2-C	37.2G	+	conformational	350-360						93.75	G355
GP2-C	10.4B	+	conformational	350-360						88.89	G583
GP2-A or C	9.7A	-	tbd	tbd						94.10	G551
GP2-A or C	8.11C	-	tbd	tbd						93.75	G551
GP2-A or C	20.5G	-	tbd	tbd						92.01	G583
GP2-D	2.10G	-	conformational	n helix or t loop						95.49	G400
GP2-D	8.4F	-	conformational	n helix or t loop						95.49	G610
GP2-D	6.6C	-	conformational	tbd						94.10	LS011
GP2-D	37.9D	-	conformational	tbd						98.21	G482
GP2-D	14.2D	-	conformational	n helix or t loop						95.83	G551
GP2-D	37.2H	-	conformational	n helix or t loop						92.71	G355
GP2-D	10.5G	-	conformational	n helix or t loop						92.01	G673
GP2-E	6.9A	-	conformational	n helix or t loop						94.79	G551
GP2-E	20.4F	-	conformational	n helix or t loop						93.4	G551
GP2-E	13.9H	-	conformational	n helix or t loop						95.49	G551
GP2-E	3.6H	-	conformational	n helix or t loop						92.01	G355
Tbd	8.9F	+	quanternary	tbd						92.01	LS011

Table 2. Selected LASV Glycoprotein huMAbs isolated from human survivors of Lassa fever in Sierra Leone.

Group	huMAb	Neut-tralizing	Epitope type	Epitope (amino acids)	V	J	CDR lengths	Seq CDR-3_L3	Mutation	patient
GP1-A	19.7E	+	conformational	111-117	Proprietary Info				94.62	G583
GP1-B	21.6 G	-	conformational	121-133					98.26	G355
GP1-B	2.4F	-	conformational	122-133					98.97	G583
GP1-C	5.11A	-	tbd	tbd					92.83	G583
GP1-C	36.7D	-	tbd	tbd					96.77	G355
GP1-C	19.5A	-	conformational	tbd, GP1 dimer					94.62	G583
GP1-C	21.5F	-	conformational	tbd					94.62	G355
GP1-C	3.3B	-	conformational	126-133					tbd	G502
GP1-C	5.6H	-	conformational	126-133					97.85	G583
GP1-C	6.7B		tbd	tbd					97.22	G583
GP1-BC	18.5D	-	conformational	121-133					97.85	G617
GP1-BC	39.3G	-	conformational	121-133					98.61	G355
GP1-BC	9.9F	-	tbd	tbd					91.76	G673
GP1-BC	37.4E	-	conformational	121-133					94.44	G355
GP1-?	12.1F	+	tbd	tbd					92.47	G448
GP2-A1	37.2D	+	conformational	270-278					94.27	G355
GP2-A1	25.10C	+	conformational	270-278					93.19	G355
GP2-A1	36.1F	+	conformational	270-278					93.62	G355
GP2-A2	37.7H	+	conformational	364-370; 377-388					94.10	G355
GP2-A2	25.6A	+	conformational	364-370; 377-388					tbd	G355
GP2-A2	36.9F	+	conformational	364-370; 377-388					63.64	G355
GP2-A2	2.9D	+	conformational	364-370; 377-388					98.99	G355
GP2-B	22.5D	-	linear	300-315					96.94	G355
GP2-B	4.1F	-	linear	303-309					93.26	G355
GP2-B	8.8B	-	linear	303-309					96.22	G400
GP2-C	37.2G	+	conformational	350-360					96.45	G355
GP2-C	10.4B	+	conformational	350-360					87.81	G583
GP2-A or C	9.7A	-	tbd	tbd					94.10	G551
GP2-A or C	8.11C	-	tbd	tbd					93.75	G551
GP2-A or C	20.5G	-	tbd	tbd					92.11	G583
GP2-D	2.10G	-	conformational	n helix or t loop					94.98	G400
GP2-D	8.4F	-	conformational	n helix or t loop					95.49	G610
tbd	6.6C		tbd	tbd					90.78	LS011
	37.9D		tbd	tbd					98.21	G482
GP2-D	14.2D	-	conformational	n helix or t loop					92.47	G551
GP2-D	37.2H	-	conformational	n helix or t loop					93.27	G355
GP2-D	10.5G	-	conformational	n helix or t loop					93.06	G673
GP2-E	6.9A	-	conformational	n helix or t loop					96.06	G551
GP2-E	20.4F	-	conformational	n helix or t loop					94.27	G551
GP2-E	13.9H	-	conformational	n helix or t loop					97.49	G551
GP2-E	3.6H	-	conformational	n helix or t loop					97.37	G355
Tbd	8.9F	+	quanternary	tbd					94.10	LS011

MILESTONE 2: Map linear or conformational B cell epitopes using mutant and wild-type recombinant LASV proteins, and overlapping synthetic peptides (Tulane).

Original Goals:

- Cross-competition assays that place MAbs into groupings.
- Prepare dicysteine linkage maps of LASV GP1, GP2, GPC, NP and Z.
- Derive epitope specific ELISA.
- Prepare GP, NP and Z MAb linkage maps.
- Establish cross-reactivities in B cell epitopes between Old/New World arenaviruses.
- Measure MAb binding to LASV proteins by Biocore, etc.
- Quantify reactivity of sera and MAbs to synthetic peptides.

OVERVIEW OF CONTRACT DELIVERABLES ACHIEVED IN MILESTONE 2.
We fulfilled each of the goals of Milestone 2.

- Cross competition assays of LASV GP1 and GP2 huMAbs placed the antibodies into useful linkages facilitating epitope mapping.
- We used X-ray crystallography to solve the structures of arenavirus GP, NP and Z, thus obviating the need to prepare dicysteine linkage maps (Milestone 3).
- We derived epitope specific ELISA and epitope mapped the huMAbs using mutant and wild-type recombinant LASV proteins, and overlapping synthetic peptides.
- We mapped the location of epitopes for the known completion groups of the huMAbs on the crystal structure of arenavirus GPC (LCMV prefusion form). As directed by the advisory committee we focused on LASV GP.
- Cross-reactivities in B cell epitopes between Old/New World arenaviruses have also been established. The majority of GP1 huMAbs do not cross-react with LCMV another Old World arenavirus. None of the GP1 antibodies to date cross react with Machupo or Junin virus GP1. A majority of the GP2 huMabs do cross-react with LCMV, and a limited number of these cross-react with Machupo and Junin virus GP2.

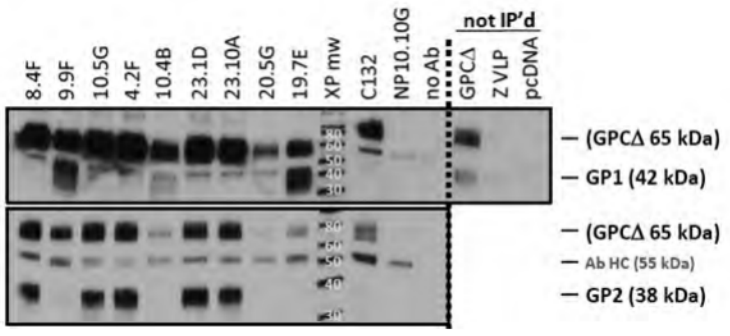


Fig. 5. The huMabs indicated were used as capture antibodies in immunoprecipitation studies employing lysates from cells expressing LASV GPs (GPC, GP1 and GP2) . Immunprecipitated proteins were then resolved by SDS-PAGE, transferred to nitrocellulose and western blotting performed with antibodies to either GP1 (upper pannel) or Gp2 (lower panel). GP1 and Gp2 detection antibodies also recognize GPC by western blotting. Specficities of the huMabs are summarized in Table 1.

- We have measured huMAb binding to LASV proteins by Biocore.
- We have quantified reactivity of sera and MAbs to synthetic peptides and demonstrated that most huMabs recognize conformational epitopes. Tree linear epitope have been identified. One of the linear epitopes is an immunodominant epitope.

Cross-competition assays that place MAbs into groupings.

To date we have identified, cloned and expressed over 120 LASV lineage IV LASV GP specific huMAbs from 15 PBMC samples. Additional huMAbs are in various stages of molecular cloning, expression or

	19.7E	10.4B	19.5A	37.4E	3.3B	5.6H	18.5D	21.6G	21.5F	5.11A	2.4F	36.7D
19.7E	++	++	-	-	-	-	-	-	-	-	-	-
10.4B	+	+	-	-	-	-	-	-	-	-	-	-
19.5A	-	-	++	++	++	++	-	-	-	-	-	-
3.3B	-	-	++	+	++	+	-	-	-	-	-	-
5.6H	-	-	++	++	++	++	-	-	-	-	-	-
37.4E	-	-	++	++	++	++	+	-	+	-	+	-
18.5D	-	-	-	-	-	-	++	-	+	-	++	-
21.6G	-	-	-	-	-	-	-	++	++	++	++	-
21.5F	-	-	-	-	-	-	-	+	++	+	++	-
5.11A	-	-	-	-	-	-	-	+	+	++	++	-
2.4F	-	-	-	-	-	-	-	+	++	+	++	-
36.7D	-	-	-	-	-	-	+	+	++	+	++	++

Fig. 6. Cross-competion studies with huMAbs to LASC GP1 of lineage IV.

purification. We have assessed antibody specificity by immunoprecipitation, ELISA and western blotting with reagents to detect binding to LASV GP1 or GP2. (an example of immunoprecipitation is shown in Fig. 5). GP2 reacting huMAbs are approximately 2 times more common than huMAbs reacting with GP1.

To roughly define epitope groups, we performed cross-competition assays in which MAbs were coated directly in wells of assay plates (Figs. 6,7). Meanwhile the same sets of MAbs were incubated separately for one hour with biotinylated purified LASV GPC (LASV lineage IV, provided by Erica Ollmann Saphire). These mixtures were then added to the wells coated with the different MAbs. Binding of biotin-GP was measured by incubation with peroxidase-streptavidin in the wells followed by color development with TMB-peroxide. If the MAb in solution (huMab designation in blue) competes with the coating MAb (green), we expect to see reduced absorbance which in the figure is denoted by + (weak competition) and ++ (strong competition). Data for a set of GP1 Mab competition shows that although the cross-competition patterns are perhaps not as well defined as we have observed with HIV-1 gp120 huMAbs, that it is possible to discern distinct groupings of MAbs, which show reciprocal or non-reciprocal, blocking (Fig. 2). Similar cross-competion patterns were observed for GP2 specific huMAbs to lineage IV LASV (Fig. 3). For GP1 huMAbs there are at least 3 distinct groups (GP1A-C) and for GP2 MAbs there are 5 or more groups (GP2A-E). The data give good indication of which MAbs do not compete and most likely identify spatially distinct, non-overlapping epitopes.

Derive epitope specific ELISA.

To map linear or conformational B cell epitopes we have used mutant and wild-type recombinant LASV proteins, and overlapping synthetic peptides. We have been performing mapping studies to determine critical amino acids in epitopes recognized by our panel of LASV huMAbs by making a series of mutations across a range of amino acid residues in LASV Josiah (lineage IV) GP1 and GP2. In order to perform mutagenesis and test mutants easily and rapidly, we have constructed plasmids for the expression of fusion proteins containing the LASV signal peptide, GP1 or GP2, or both, and a linear HIV-1 gp120 C terminal epitope APTKAKRRVVQREKR (designated pSP-GP1-GP2-D7). We call this linear epitope “tag” the D7 epitope after a commercially produced sheep antibody, D7324 (Aalto Bio Reagents Ltd, Dublin, Ireland), which was raised against this same site and which has been widely used in HIV research (for example: [12]).

In addition, some years ago we derived a D7 epitope-specific rhesus Mab, 2.3G, from a SHIV infected macaque. Recently a collaborator (Yongun Guan, U Maryland), engineered a molecular clone of this Mab to contain the heavy chain constant region of murine IgG2a, and the Mab is now designated JR52. The “murinized” Mab JR52 has proven to be extremely

	6.9A	20.4F	13.9H	3.6H	2.10G	8.4F	14.2D	37.2H	9.7A	37.2D	36.9F	37.2G	37.7H	20.5G	8.8B	22.5D	4.1F
6.9A	++	++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
20.4F	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13.9H	++	++	++	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3.6H	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-	-
2.10G	++	++	+	+	+	+	+	++	-	-	-	-	-	-	-	-	-
8.4F	++	++	++	++	++	++	++	++	-	-	-	-	-	-	-	-	-
14.2D	++	++	+	+	+	+	+	++	-	-	-	-	-	-	-	-	-
9.7A	+	-	-	-	-	-	-	-	++	++	++	++	++	+	-	-	-
20.5G	-	-	-	-	-	-	-	-	-	+	++	++	++	++	-	-	-
37.2D	-	-	-	-	-	-	-	-	-	+	++	++	++	+	-	-	-
36.9F	-	-	-	-	-	-	-	-	-	-	++	++	++	+	-	-	-
37.2G	-	-	-	-	-	-	-	-	-	-	++	++	++	+	-	-	-
37.7H	-	-	-	-	-	-	-	-	-	-	++	++	+	+	-	-	-
8.8B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	++	+
22.5D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	++	+
4.1F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++

Fig. 7. Cross-competion studies with huMAbs to LASC GP2 of lineage IV.

useful as a capture reagent for mutant fusion proteins in sandwich assays in which we detect binding of human IgG antibodies with enzyme-labeled goat anti-human IgGFc, thus avoiding reactivity with the capture reagent. In preliminary experiments all LASV fusion proteins with the C-terminal D7 tag are readily immobilized in ELISA plates coated with JR52 MAb and thus permitted detection of human antibodies binding to GP1 or GP2.

We constructed three D7 tagged expression plasmids based on LASV Josiah strain (lineage IV) LAS-SP-GP1-D7 expresses soluble GP1 very nicely. The LAS-SP-GP1-D7 fusion protein is secreted from transfected cells and is readily detected in culture fluids by all the GP1-specific MAbs we have made to date. Antigen is quite stable after freezing. Using pLAS-SP-GP1-D7 we have performed alanine-scanning mutagenesis in a portion of GP1 and will continue this approach to complete the survey of alanine substitutions in GP1.

The GP2-D7 fusion protein encoded by the pLAS-SP-GP2-D7 plasmid is also secreted but epitopes recognized by GP2-specific neutralizing MAbs do not bind to it, whereas most non-neutralizing GP2 specific MAbs bind well. Mutagenesis of LAS-SP-GP2-D7 has been limited to examining the effects of truncating GP2 by deleting amino acids from the C terminal end: GP2 aa260-333, aa260-397 and aa260-409. The parental construct, GP2-ΔTM consists of GP2 aa260-433. Each of these proteins are secreted by transfected HEK-293T cells and readily detected in detergent treated culture medium in the D7-JR52 capture ELISA.

The third plasmid construct results in expression of LASV GP1-GP2-D7 with the D7 sequence replacing the transmembrane and intracytoplasmic domain of GP2. Although this fusion protein is not well secreted, ample protein is present in transfected cells and can easily be extracted by solubilizing cells with non-denaturing detergents. Hence we use cell lysates as a source of antigen. This protein tends to be unstable with freezing and thawing perhaps due to protein aggregation. Nevertheless, we have been able to mutagenize GP2 in this construct and obtain valuable results as long as we use recently prepared detergent-treated cell extracts

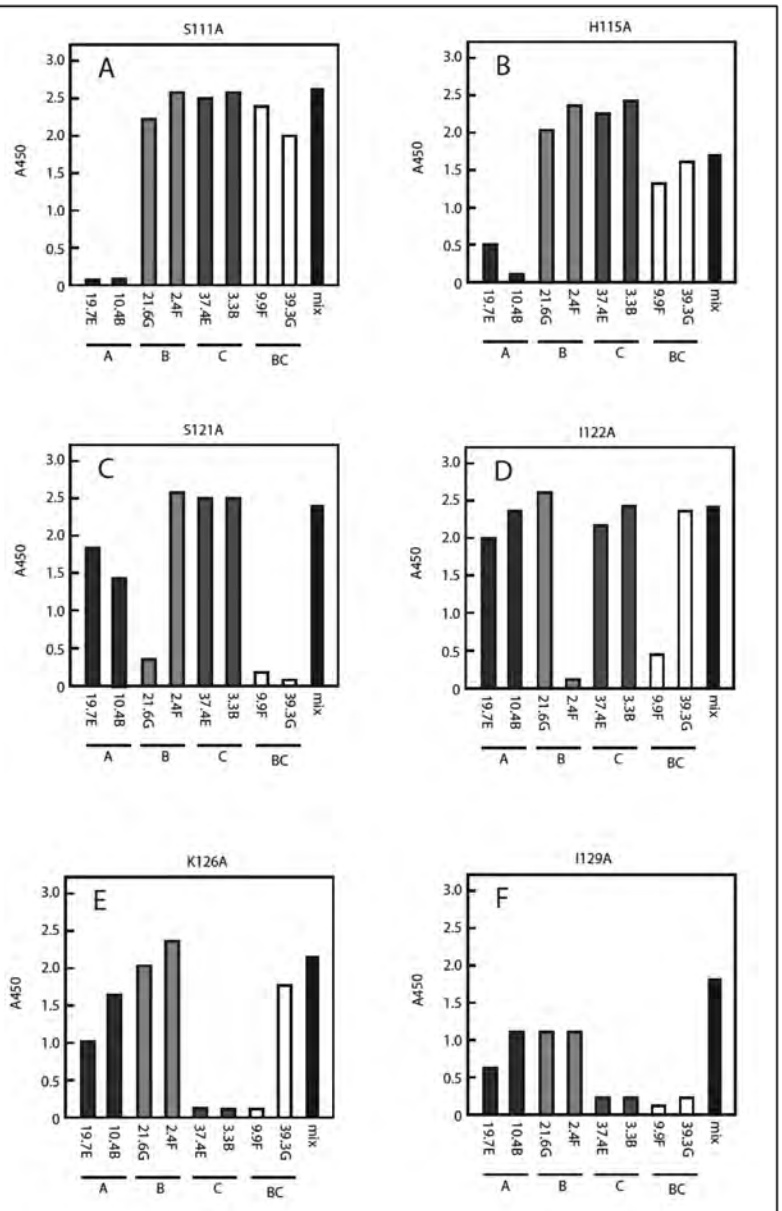


Fig. 8. Mutagenesis mapping of GP1 epitopes.

as a source of antigen. In addition to performing alanine substitution mutagenesis targeting the fusion loop and the T loop of GP2 we have also created a mutant in which the fusion loop was deleted.

To test binding of MAbs to expressed parental or mutated proteins, we coat ELISA plates with the murinized MAb JR52 and then capture D7-tagged proteins in the wells. After wells are blocked MAbs are reacted with captured proteins and their binding is detected with peroxidase-conjugated goat anti-human IgG (gamma specific). Note that the conjugate must not cross-react with murine IgG. Color is developed with TMB-H2O2, reaction is stopped with phosphoric acid and plates are read in an ELISA plate reader at 450 nm

We have performed alanine-scanning mutagenesis in GP1 and GP2 to identify critical amino acids that affect epitope recognition. In future studies we also plan to generate a series of domain swaps between LCMV and LASV to find out which residues in LCMV explain a lack of reactivity with LCMV GPC. We are not stuck on alanine substitutions and will use other amino acid substitutions in the future. Results of the scanning mutagenesis fine mapping in conjunction with peptide scanning are described for the various epitope groups.

LASV GP1-A-C epitope groups.

LASV GP1 huMabs break into 3 cross competition groups designated GP1-A-C (Tables 1, 2, Fig. 6). Certain huMAbs in groups B and C cross-compete with huMabs in the both groups suggesting that the “epitope for GP1B and C overlaps or is in a close vicinity on intact LASV GPC. To date we have not detected any huMAbs that bind to linear peptides of LASV GP1. Scanning mutagenesis clearly identifies key amino acids that contribute to the epitope represented by huMabs in the three GP1 groups (see below). 19.7E and 10.4B, which have low neutralizing activity but nevertheless provided protection in guinea pig models of LF do not

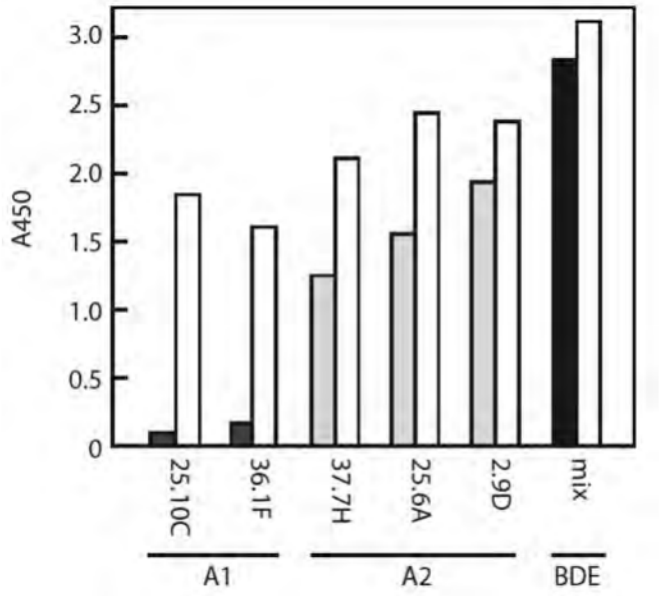


Fig. 9. huMAb binding to fusion loop deletion mutants of LASV GP.

bind to constructs containing the S111A and H115A mutations (Fig. 8A, B). Additional mutagenesis studies indicate that the GP1-A epitope spans GP1 amino acids 111-117. Studies using LASVpseudoparticles (LASVpp) constructed with LASV GPs of different lineages I-IV, confirm the location of the GP1-A epitope (Milestone 6). The mutagenesis results with 10.4B clearly place it in the same group as 19.7E. However, we have obtained conflicting DXMS results with 10.4B, which indicate that it is in group GP2-C. We expect that further studies will sort out these conflicts.

Scanning mutagenesis of huMabs in groups GP1-B (for example: 21.6G and 2.4F) and C (37.4E and 3.3B) indicate that these epitopes are represented by key amino acids 121-125 and 125-133, respectively. Certain antibodies (for example: 9.9F and 39.3G) failed to bind to constructs containing mutations across the entire region from 121-133 and these huMabs are placed in both groups, GP1-BC.

LASV GP2A1 and A2 epitope groups.

LASV GP2 huMabs break into 5 cross competition groups designated GP-1A-C (Tables 1, 2 and Fig. 7). Group GP2-A appears to represent two epitopes in close proximity on intact GPC, hence the ability of huMAbs in group GP2-A to cross-compete. The epitope of huMAbs of group A1 (examples: 25.10C, 36.1F and 37.2D) involved the GP2 fusion loop, which is near the amino terminus of GP2 and mediates virion:cell membrane fusion. A GP construct that deletes the fusion loop fails to bind GP2A1 antibodies (Fig. 9). However, huMAbs of group GP2-A2 (examples: 37.7H, 25.6A and 2.9D) bind the fusion loop deletion constructs at levels that are reduced, but remain comparable to binding to the wild-type non-deleted construct (Fig. 9).

The huMAbs of other groups also bind to the fusion loop deletion construct with similar efficiency as the wild-type construct indicating that the deletion of the fusion loop does not grossly perturb the overall structure of LASV GP. However, we are cognizant that such large deletions can perturb overall protein structure and we will utilize additional means to map the GP2-A epitopes. Scanning mutagenesis confirms that mutation of amino acids in the fusion loop prevent binding of group GP2-A1 (examples: 25.10C, 36.1F and 37.2D), but do not block binding of group GP2-A2 antibodies (examples: 37.7H, 25.6A and 2.9D) or GP2-B-E antibodies (Fig. 10). Converting amino acids 273-276, 279-281, 285-287 or 291-293 to alanines significantly reduces binding of the GP2-A1, but not the GP2-A2 antibodies. These results indicate that the GP2-A2 epitope encompasses amino acids 270-278 and other residues in the LASV GP2 fusion loop. Studies using LASVpp constructed with LASV GPs of different lineages I-IV, confirm the location of the GP2-A1 epitopes (Milestone 6). These results also indicated that antibodies of groups GP2-A2 (examples: 37.7H, 25.6A and 2.9D) and C (example: 37.2G)

that cross-compete with the GP2-A1 antibodies (Fig. 7) bind to epitopes outside the fusion loop.

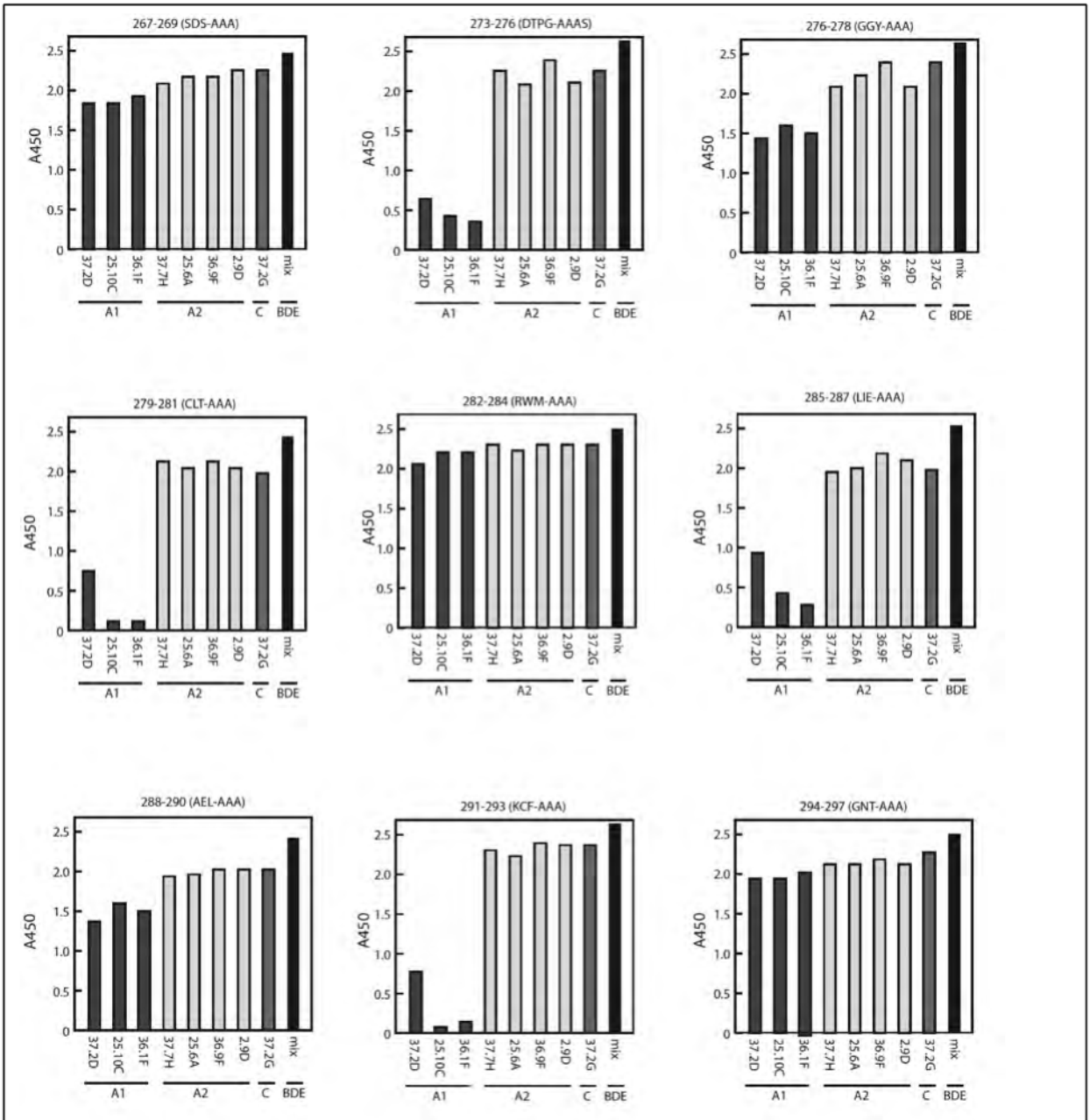


Fig. 10. Mutagenesis mapping of fusion loop epitope GP2-A1.

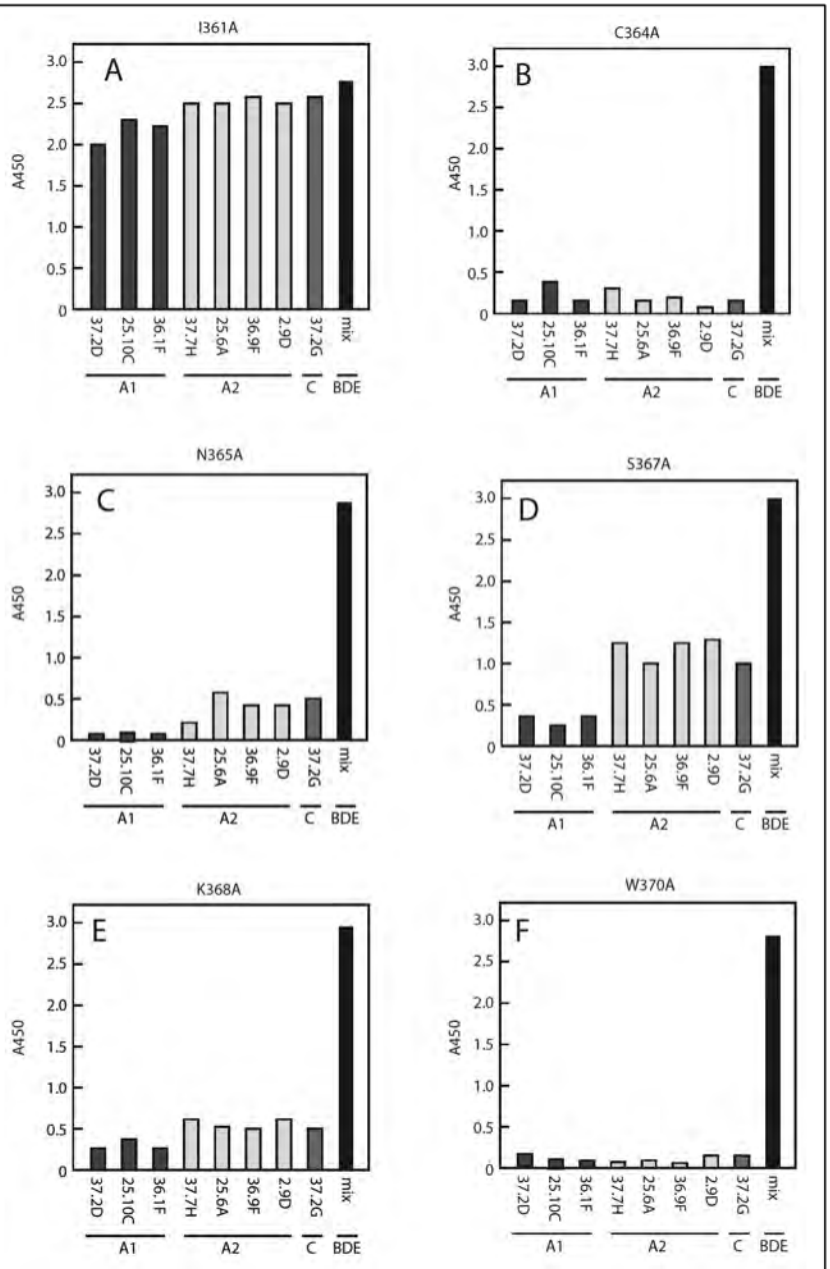


Fig. 11. Binding of huMabs to conformational epitopes A1, A2 and C is blocked by mutations in the T loop.

Additional scanning mutagenesis identified residues in or near the T-loop of GP2 as the location of the epitopes of GP2-A2 and C huMAbs. Mutations C-364A, N365A, S367A, K368A and W370A perturb the binding of group A1, A2, and C antibodies, without preventing binding of huMabs of groups B, D or E. Given the close proximity of the fusion loop to residues in the T loop (see Milestone 3) and the clear results of the deletion (Fig. 10) and mutagenesis studies (Fig. 11) the failure of the GP2-A1 antibodies to bind to these T loop mutant constructs is likely due to perturbation of the confirmation of the fusion loop by the alteration in these key residues of the adjacent T-loop. DXMS analysis indicates that the epitope of GP2-C antibody 37.2G is directly adjacent to the residues mutated in Fig. 7, hence accounting for disruption of this conformational epitope. DXMS and studies with LASVpp of lineage I-IV (Milestone 6) and mutagenesis suggest that key residues of the GP2-A2 epitope include amino acids 364-370 and 377-388. Additional studies using scanning mutagenesis and deletions are in process to confirm this hypothesis. Antibody 13.4E (group GP2-A2a) recognizes a linear epitope SKYWYLN (367-373) that overlaps with the GP2-A2 epitope in the T-loop.

LASV GP2-B epitope group.

The mapping of LASV huMabs of group GP2-B is based on linear peptide scanning data. 61 different overlapping 15-mer peptides were designed, spanning the entire length of LASV GPC. These peptides were purchased from ProImmune (thinkpeptides). MALDI-TOF Mass Spectrometry (MS) is performed on 100% of the sample. Each peptide met both the MS

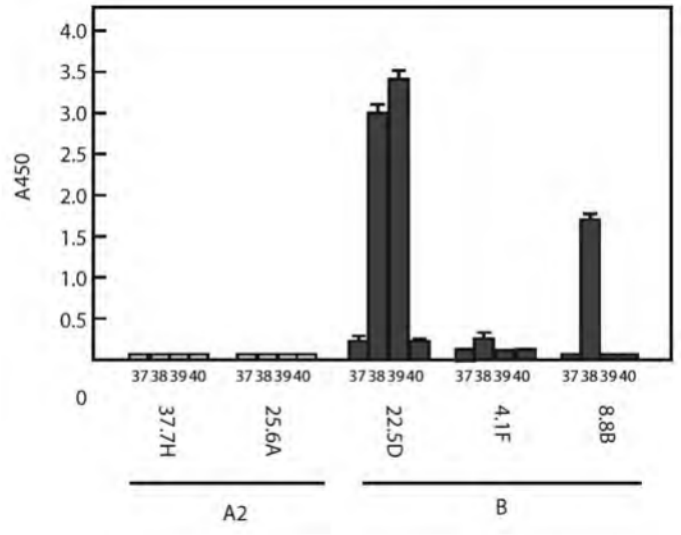


Fig. 12. Binding of GP2-B huMabs to linear peptides. Binding of 4.1F to peptide 38 is low but reproducible.

analysis and final gross weight criteria to pass QC. Each peptide was dissolved in HFIIP, allowed to sit overnight at room temperature, and then sonicated for 15min. Prior to use, the peptides were then lyophilized back into powder form and resuspended in 40% acetic acid and water. The peptides were again sonicated and allowed to sit overnight at room temperature. If the peptides were not 100% dissolved, a 10% ACN and water solution was added, and the peptides were then re-sonicated prior to use. Nunc PolySorp (Nunc) 96-well plates were coated with peptide at a final concentration of 200 ng per well in Sodium Bicarbonate Coating Buffer, pH 9.5. Plates were incubated overnight at 4°C and washed three times with PBST. Plates were then blocked for 60 min with 200 µL of blocking buffer consisting of 5% milk in PBST, then washed as above. A 1 µg per ml concentration of LASV mAb mix in blocking buffer was added at a final volume of 100 µL/well. The plates were incubated for 1 h at 37°C, then washed as above. Detection was performed with 100 µL/well of HRP-conjugated goat α-human IgG antibody reagent (KPL)) diluted to 1:2500 in blocking buffer. After 1 h incubation, 100 µL/well of TMB substrate (Sigma) was added, and the plates were incubated for 20 min. The reaction was stopped by adding 100 µL/well of TMB stop solution (KPL) and read at 450 nm in a BioTek fluorescence reader.

Contrary to our initial expectations there were few huMabs that reacted to linear peptides of LASV GP. The exception were three huMabs, 22.5D, 4.1F and 8.8B in cross-competition group GP2-B that react with peptide 38 (AVAKCNEKHDEEFCD, Fig. 12). Binding of 22.D to peptide 38 is strongest; 8.8B is the second strongest. The binding of 4.1F is weak, but highly reproducible. 22.5D also reacts strongly with peptide 39 and weakly with peptides 37 and 40. Key amino acids of epitope representing group GP2-B are 303-309. Site-directed mutagenesis studies and studies with variant peptides are in process to confirm the key residues in epitope GP2-B.

LASV GP2-C epitope group.
The mapping of LASV huMabs of group GP2-C is based on DXMS data discussed in Milestone 4.

LASV GP2D, E and F epitope groups.
LASV GP2 D (examples: 6.7A, 13.9H, 20.4F, 3.6H) and GP2-E (2.10G, 8.4F, 14.2D 37.2H) huMAb likely represent overlapping epitopes as the huMAb in group GP2-D block binding of both GP-2D and E antibodies. GP2-E antibodies block other GP2-E antibodies, but do not block GP2-D antibody binding to GP2. Fine mapping of GP2-D and E epitopes is in process. GP2 truncations of different lengths were constructed on LASV-SSP-GP2-D7 in order to approximate the location: GP2 aa260-333, aa260-397 and aa260-409, and aa260-433. The

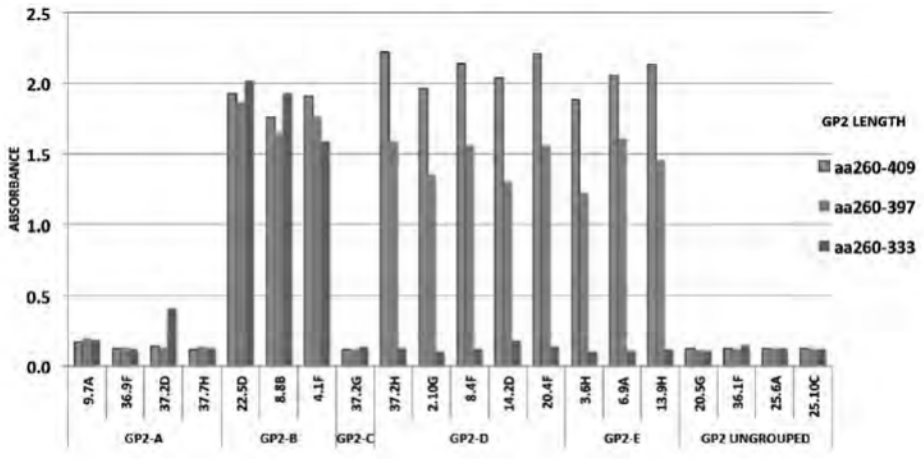


Fig 13. Binding of huMabs of the indicated cross-competition groups to proteins expressed from LASV-SSP-GP2-D7 constructs of different lengths.

longest (a.k.a. GP2ΔTM) ends just proximal to beginning of the putative transmembrane region of GP2 at aa333. Each of these proteins are secreted by transfected HEK-293T cells and readily detected in detergent-treated culture medium in the D7-JR52 capture ELISA. The cross-competing GP2 neutralizing huMAbs of competition group GP2-A, do not bind to any of the GP2-D7 fusion proteins, including the longest construct designated GP2ΔTM (Fig. 13). Were it not for the lack of reactivity with GP2DTM we might have inferred that these huMAbs bind in the membrane proximal external region (MPER). However, we believe that these antibodies are binding to the fusion loop near the amino terminus and that the SSP is perturbing the epitope. A similar construct with V1/2 instead of D7 gave a similar pattern (not shown). 37.2 G, a GP2-C antibody, also failed to bind the construct. It is possible that this region of the molecule assumes a different configuration in this construct than in the virion GP2 configuration to which it must bind. The huMAbs of cross-competition group GP2-B a linear epitope reacted with all three truncated GP2 proteins. Competition groups GP2-D and GP2-E reacts with both the two longer truncated proteins (aa260-397, and aa260-409), as well as with GP2ΔTM), but reactivity with the shortest protein (aa260-333) is lost. These results locate the D and E epitopes in N helix or T loop amino acids 333-397. Scanning mutagenesis studies are in process to identify the epitopes recognized by huMabs of groups GP2-D and E. Antibody 24.6C recognizes a linear epitopes (GP2-F) DDIEQQADNMITEML (401-415).

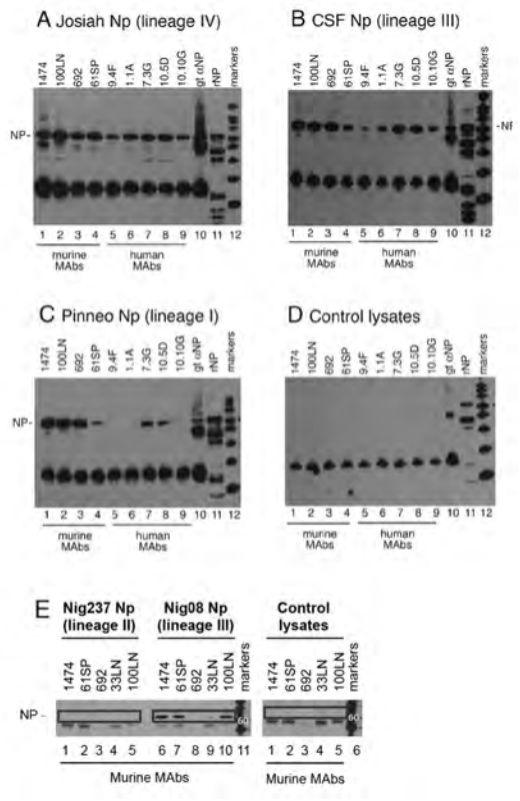


Fig. 14. Characterization of murine and human NP mAbs using mammalian cell expressed NP from Josiah (Sierra Leone strain), and 4 Nigerian strains (Pinneo, CSF, Nig237, Nig08). MAbs used in current lineage IV LFI do not IP lineage II NP (E).

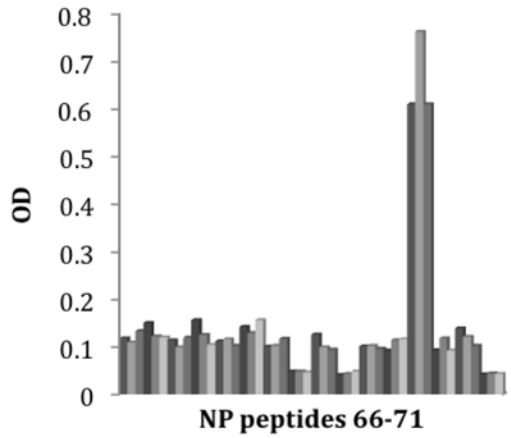


Fig. 15. Reactivities of human monoclonal antibody 10.10G to a peptide representing a potential linear B cell epitope in humans near the carboxyl terminus of the LASV NP proteins. Each of triplicate determinations is shown.

NP huMAbs The number of LF cases in Nigeria has increased sharply in recent years with many cases reported from States not traditionally thought to have a high incidence of the disease. We have assessed the ability of immunodiagnostics based on the lineage IV LASV (Josiah) to detect Nigerian strains of LASV. Whereas the immunoassays are highly sensitive and specific for strains of LASV circulating in Sierra Leone and surrounding countries it did not have high sensitivity and specificity for strains of LASV circulating in Nigeria and are currently being reconfigured to increase the lineage specificity. These results suggest that conserved epitopes that permit the use of two murine MAbs in a lateral flow immunoassay for LASV lineage IV are not present or accessible in NP of Nigerian strains (primarily lineage II). To test this hypothesis we expressed NP from Josiah (Sierra Leone strain), and 4 Nigerian strains (Pinneo CSF, Nig237-2010, Nig08-A18-2008). LASV Josiah is of lineage IV, LASV Pinneo is Lineage I, Nig237-2010 is of lineage II, and CSF and Nig08-A18-2008 are of lineage III. Lineage III is genetically closer to lineage IV than either are to lineage I. All MAbs (mouse and human) pull down Josiah NP, the strain they were generated against (Fig. 14). Both murine MAbs raised to lineage IV LASV and human MAbs derived from Sierra Leonean LF survivors pull down NP from Josiah, but are not as reactive with NP from Nigerian strains (Fig. 10A-D). Human mAbs 9.4F and 1.1A reacted poorly with Pinneo NP (lineage I), and both showed somewhat better reactivity with CSF NP (Fig. 14B,C). Similarly, 4 of 5 murine MAbs pulled down Nig08-A18-2008 NP to some degree, but all failed to immunoprecipitate lineage II Nig237-2010 NP (Fig. 14E). A potential linear epitope has been identified for the human monoclonal antibody designated 10.10G (Fig. 15).

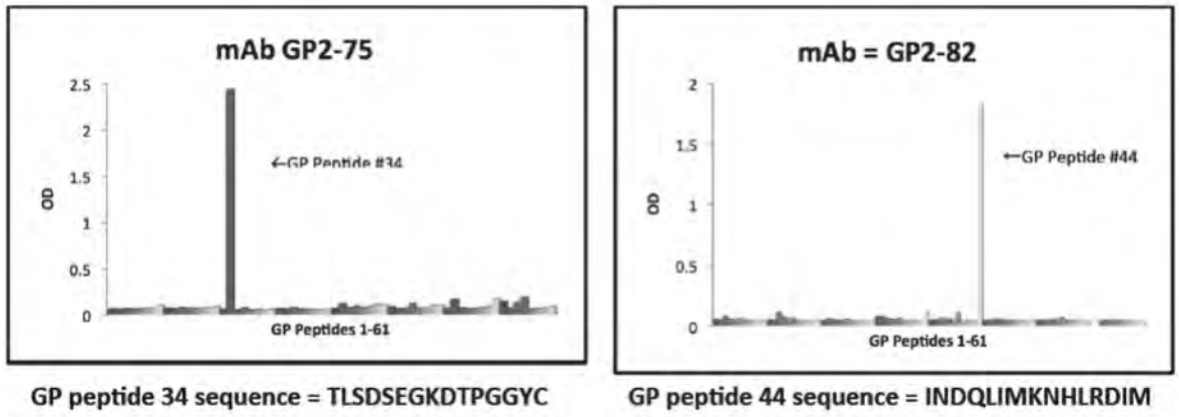


Fig. 16. Pepscan determination of epitopes recognized by mouse monoclonal antibodies to LASV GP2. 61 overlapping peptides representing the entire coding squence of GP2 were coated on ELISA plates. Each GP2 MAb reacted repeatedly and specifically to a single peptide. Binding could be competed by soluble peptide (not shown).

Mouse monoclonal antibodies.

Although not a mandate of the contract we have also mapped a limited number of mouse monoclonal antibodies to LASV proteins. It is of interest that murine MAbs recognize several potential linear epitopes on LASV GP, in contrast to the huMabs that with the exception of one group GP2-B are conformational. The corresponding amino acid sequences for these potential

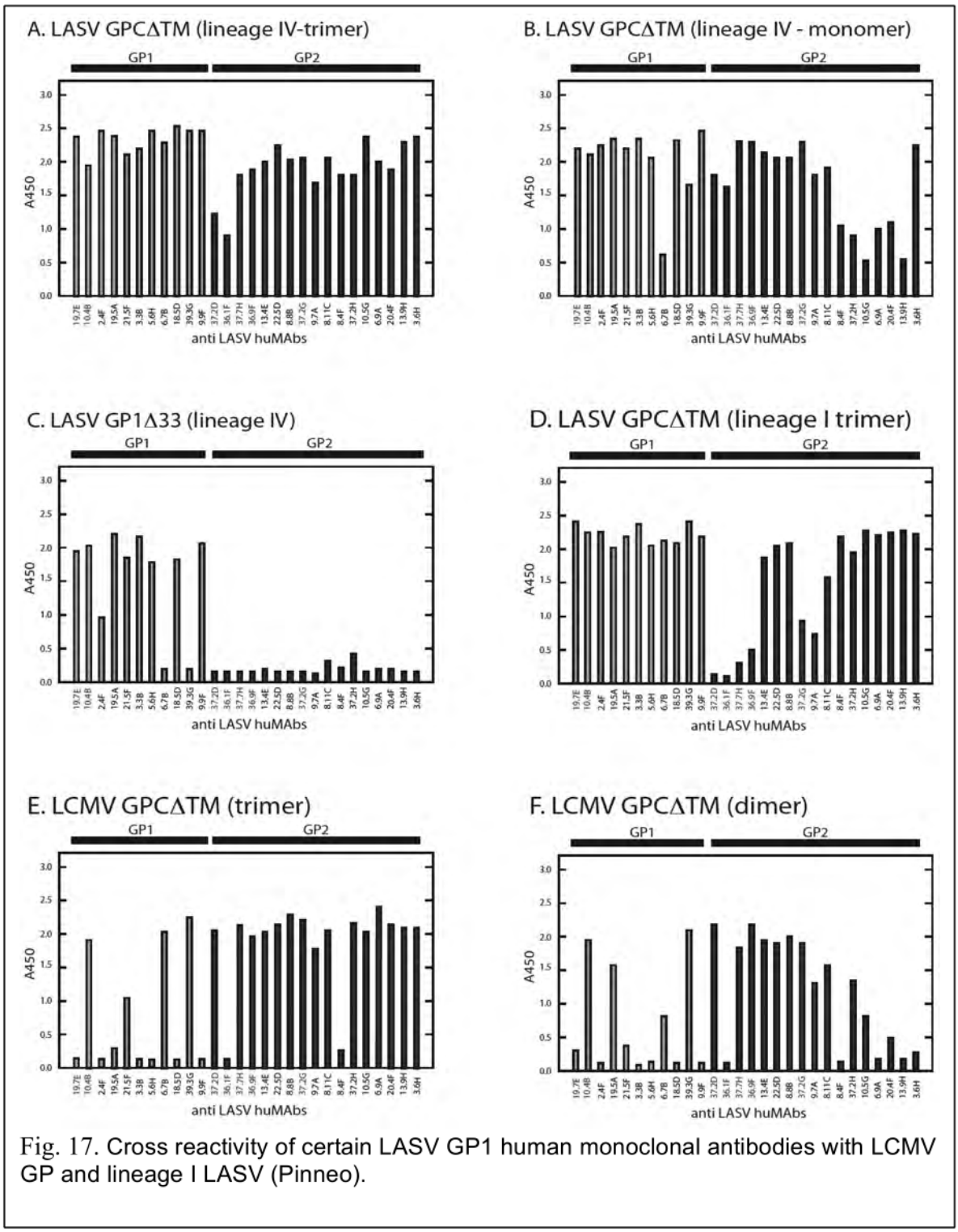


Fig. 17. Cross reactivity of certain LASV GP1 human monoclonal antibodies with LCMV GP and lineage I LASV (Pinneo).

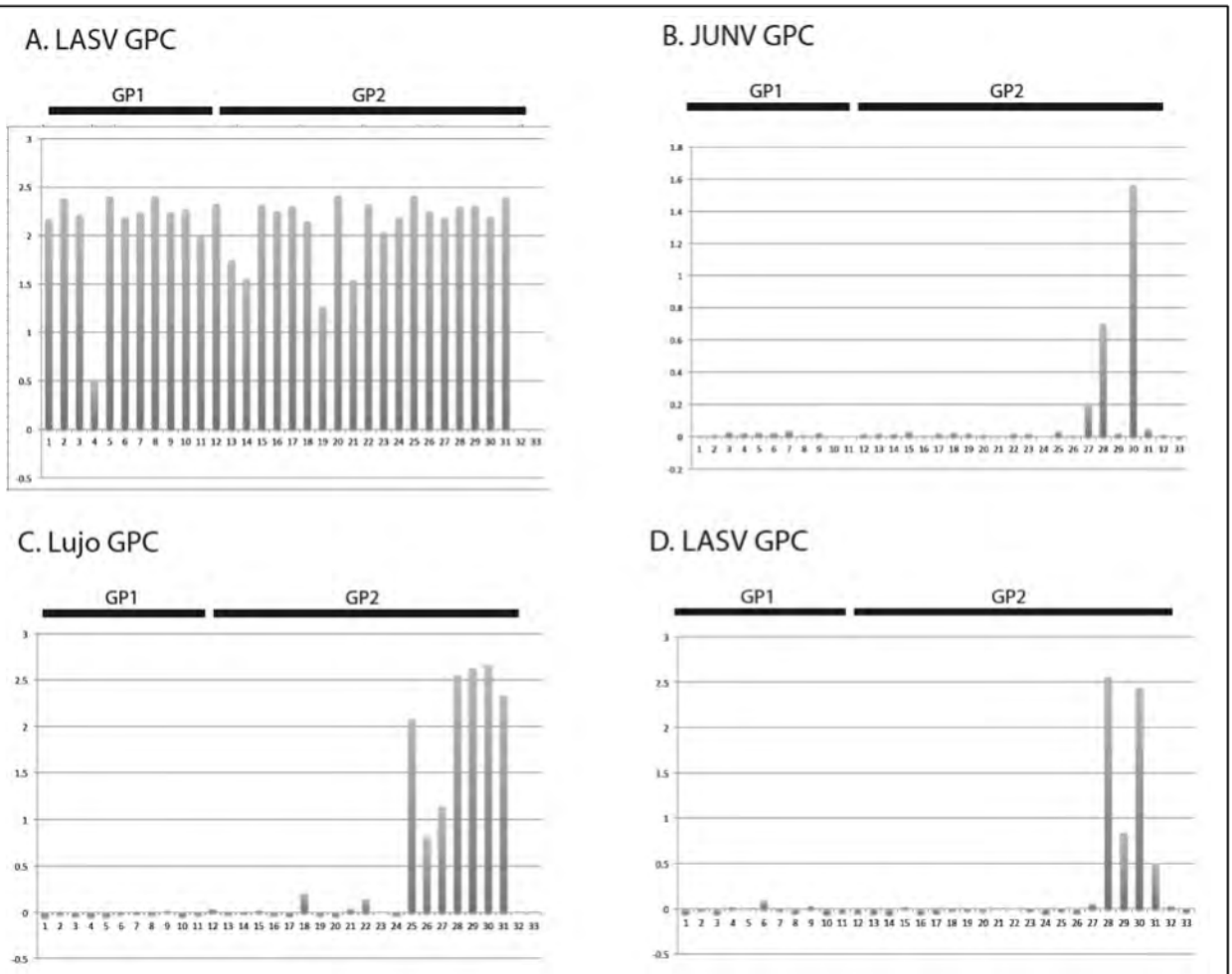


Fig. 18. Cross reactivity of selected LASV human monoclonal antibodies with Lujo, JUNV and MACV GP2.

epitopes are represented by linear peptides with the sequences TLSDSEGKDTPGGYC (fusion loop) and INDQLIMKNHLRDIM (N helix) (Fig. 16), and TGRTSLPKCWLVSNG (T loop, not shown). The reservoir of LASV is a rodent and human and mouse antibodies differ in a number of parameters including the length of the CDRH3 domains.

Cross-reactivities in B cell epitopes between Old/New World arenaviruses.

Only 4/14 of the huMAbs with LASV GP1 specificity tested were cross-reactive with LCMV GP (Fig. 17E). A majority of huMAbs (21/33) with GP2 reactivity were cross-reactive with lymphocytic choriomeningitis virus (LCMV) GP (Fig. 17E). GP2 huMAbs 6.9A, 8.8B, 13.4E, 20.4F, 22.5D, 23.2E and 37.2H reacted to Lujo GPC (Fig. 18). Two antibodies 8.8B and 22.5D, both of group GP2-B, reacted to JUNV GP. These two antibodies plus 13.4E (linear epitope) also react to Macupo virus GP. The key residues of the linear epitope of the GP2-B antibodies are NEKHDEEF. This GP2-B epitope sequence in JUNV and MACV GP is NLNHDSEF and NQNHDSEF, which is conserved in some but not all residues.

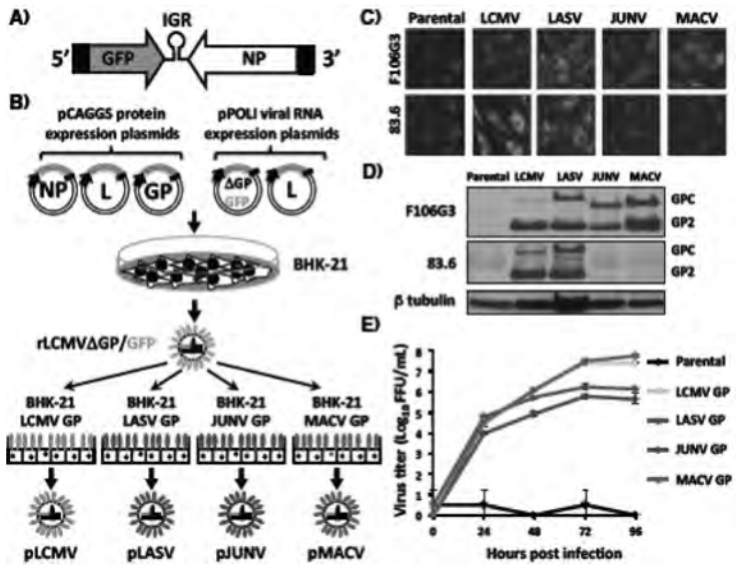


Figure 19. Generation of GP-pseudotyped rLCMV**Δ**GP/GFP. A) Schematic representation of the GFP/NP vRNA S segment: Black boxes: non-coding regions. IGR: intergenic region. B) Generation of GP-pseudotyped rLCMV**Δ**GP/GFP: BHK-21 cells were co-transfected with pCAGGS (NP, L, and GP) and pPOLI (L and S **Δ**GP/GFP) plasmids[40]. At 72 hours post-transfection, BHK-21 cells constitutively expressing the indicated arenavirus GPs were infected with supernatants to generate GP-pseudotyped rLCMV**Δ**GP/GFP[40]. C-D) Characterization of GP-expressing cell lines: Parental and GPexpressing BHK-21 cells were tested for GP expression by immunofluorescence (C) and Western blot (D) with the cross-reactive GP2 F106G3 (OW and NW) and 83.6 (OW) MAbs. E) Growth properties of rLCMV**Δ**GP/GFP: Parental and GP-expressing BHK-21 cells were infected with rLCMV**Δ**GP/GFP (moi = 0.1) and supernatants collected at the indicated times post-infection were titrated by GFP expression (FFU/ml) on GP-expressing cells. Infections were performed in triplicates. Mean values and standard deviation are shown. *Data by Luis Martinez and Juan Carlos de la Torre.*

Rescue of rLCMVΔGP/ GFP pseudotyped with arenavirus GPs of interest. Additional studies with the LASV huMAbs have been undertaken by our collaborators Luis Martinez, University of Rochester Medical Center and Juan Carlos de le Torre (Scripps). These studies confirm cross-reactivities beteen LASV and other Old World arenaviruses. To generate a construct used in these studies rLCMV**Δ**GP/GFP, BHK-21 cells were co-transfected with the viral (v)RNA expression pPOLI-S **Δ**GP/GFP (Fig 19A) and pPOL-L plasmids, together with pCAGGS protein expressing plasmids for the viral trans-acting factors NP and L required for virus replication and gene expression (Fig 19B). The incorporation into the transfection of pCAGGS expressing the GP of interest will result in the generation of the corresponding GP-pseudotyped single-cycle infectious rLCMV**Δ**GP/GFP. At 72 hours post-transfection, tissue culture supernatants were used to infect fresh monolayers of BHK-21 cells constitutively expressing OW (LCMV or LASV) or NW (JUNV or Machupo virus, MACV) GPs to generate GP-pseudotyped rLCMV**Δ**GP/GFP viruses. GP-expressing BHK-21 cell lines were characterized by IF (Fig 19C) and WB (Fig 19D) as described40. As predicted, multiplication of rLCMV**Δ**GP/GFP occurred in GP-expressing, but not parental, BHK-21 cell lines (Fig 19E).

Screen of LASV GP-specific hMAbs to identify those that cross-react with GP of strain Armstrong (ARM) of LCMV. A set of 10 LASV GP-specific huMAbs were characterized with respect their ability to recognize LCMV-ARM and LASV-JOS GP-expressing BHK-21 cells (Fig 20) and to neutralize LCMV-ARM and LASV-JOS GP-pseudotyped rLCMV**Δ**GP/GFP (Fig 21) using our GFP-based microneutralization assay40. Four hMAbs recognized both LCMV-ARM and LASV-JOS GPexpressing cells (Figs 20C and 20D) and notably hMAbs 37.2G, 37.2D, and 36.9F (Fig 2C) were able to neutralize rLCMV**Δ**GP/GFP pseudotyped with either LASV-JOS or LCMV-ARM GPs (Fig 21). These results demonstrate the ability of LASV GP-specific hMAbs to cross-react with and neutralize LCMV ARM.

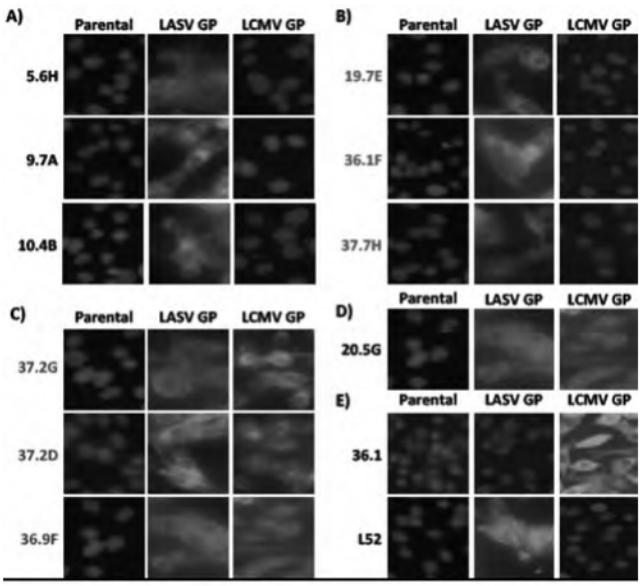


Figure 20. Cross-reactivity of LASV GP-specific huMAbs with LCMV ARM: Immunofluorescence of parental, LASV and LCMV GP-expressing BHK-21 cell lines with the indicated hMAbs. Blue: nuclei staining. A-B) huMAbs that recognize LASV GP and do not neutralize (A) or neutralize (B) pLASV rLCMV**Δ**GP/GFP (Fig 3). C-D) huMAbs that recognize both LASV- and LCMV-GP and neutralize (C) or do not neutralize (D) pLASV and pLCMV rLCMV**Δ**GP/GFP (Fig 3). E) Positive control mouse MAbs for LCMV (36.1) and LASV Joshia (L52) GPs. See Fig. 23 for compete dataset. *Data bv Luis Martinez and Juan Carlos de la Torre.*

C1.3. In vivo neutralization activity of LASV GP-specific hMAbs that cross-react with and neutralize LCMV-ARM. To assess whether the ability of hMAbs to neutralize in vitro LCMV-ARM and LASV-JOS GPpseudotyped rLCMV**Δ**GP/GFP (Fig. 21) correlated with their neutralizing activity in vivo, Drs. Martinez and de le Torre examined the effect of treatment with hMAbs 37.2D (neutralized in vitro both LCMV-ARM and LASV-JOS GPs) and 19.7E (neutralized in vitro only LASV-JOS GP) on rLCMV-ARM/WT and rLCMV-ARM/LASVGP multiplication in mice. To facilitate the experimental readout we used the immunosuppressive Clone 13 (Cl-13) strain of LCMV. Infection (i.v.) of B6 WT mice with a high dose (**≥**106 PFU) of Cl-13 causes impaired dendritic and T-cell functions that results in a transient generalized immunosuppression and establishment of a persistent infection whose parameters are well established42. Virus clearance takes place between days 60-100 p.i. and requires a functional B cell response. They predicted that hMAbs exhibiting in vivo neutralizing activity would prevent the establishment of Cl-13 persistence. They confirmed first that mutation F260L in GP1 of Cl-13 did not affect its recognition by hMAB 37.2D, then treated mice with the indicated huMAbs (20 mg/Kg; i.p.) (Fig 22) and subsequently infected them with either rCl-13/WT or rCl-13/LASV-GP(mCD) (2 x106 pfu; i.v.). rCl- 13/LASV-GP(mCD) was used because this recombinant virus contains a version of LASV-JOS GP that incorporates mutations C459K and K461G within in its cytosolic domain, a region no involved in recognition by Abs, which enhances persistence in mice43. Mice treated with hMAb 37.2D prevented persistence by both rCl-13/WT and rCl-13/LASV-GP(mCD), but huMAb 19.7E prevented only rCl-13/LASV-GP(mCD) persistence. These results demonstrate the ability of LASV GP-specific hMAbs to neutralize LCMV ARM in vivo and their potential use to control LCMV infection.

Measure MAb binding to LASV proteins by Biocore.

We have measured the binding kinetics of a subset of the huMAbs directed to LASV GP and NP (Table 3). All analysis was done on a Biacore 3000. A GE Healthcare Sensor Chip CM5 was coated with 25ug/ml Human IgG. Interaction analysis was performed by injection of LASV MAb antibodies (200nM) to produce a response between 120 (Rmin)-185(Rmax) "response units." Flow was 10ul/min in a HEPES-EP buffer, pH 7.5. Once the Mabs were covalently immobilized, the flow rate was changed to 30ul/min. Varying concentrations of LASV-NP protein were then injected and kinetic parameters determined. A 1:1 Langmuir binding curve was used.

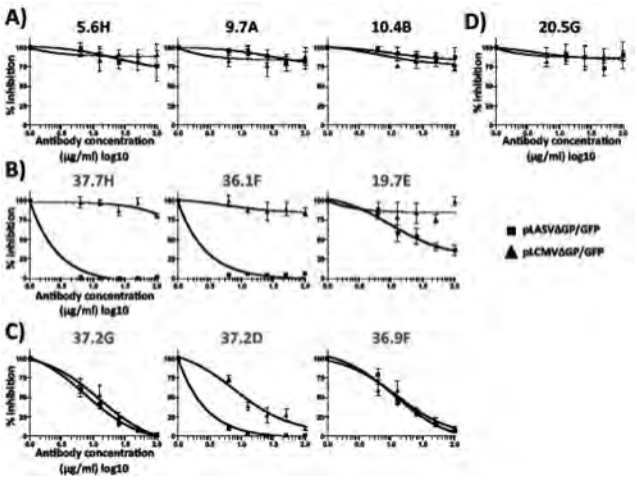


Figure 21. Neutralizing activity of LASV GP-specific hMAbs with LCMV ARM: GP-pseudotyped LASV and LCMV rLCMV**Δ**GP/GFP viruses were preincubated with 2 fold-dilutions of LASV GP-specific hMAbs (starting concentration of 5 ug) for 90 min at 370C. The virus-antibody immune complex mixture was used to infect LCMV GP-expressing cells (96 plate format, triplicates). Virus neutralization was quantified using a GFP microplate reader, 72 h p.i.. Virus infection in the absence of hMAbs was used as 100%. Mean values and standard deviation are shown. A) hMAbs that specifically recognize LASV GP (Fig 2) but do not neutralize GPpseudotyped LCMV or LASV rLCMV**Δ**GP/GFP (5.6H, 9.7A, 10.4B, and 20.5G). B) hMAbs that specifically recognize LASV GP (Fig 2) and neutralize GP-pseudotyped LASV rLCMV**Δ**GP/GFP (37.7H, 36.1F, and 19.7E). C) hMAbs that recognize both LASV- and LCMV-GP (Fig 2) and neutralize GPpseudotyped LCMV and LASV rLCMV**Δ**GP/GFP (37.2G, 37.2D, and 36.9F). D) hMAb that recognize both LASV- and LCMV-GP (Fig 2) but do not neutralize GP-pseudotyped LCMV or LASV rLCMV**Δ**GP/GFP (20.5G). See Fig. 24 for compete dataset. *Data by Luis Martinez and Juan Carlos de la Torre.*

In general average K_D values of most human antibodies are in the low micromolar (10^{-6}) to nanomolar (10^{-7} to 10^{-9}) range. High affinity antibodies are considered to have K_D values n the low nanomolar range (10^{-9}) and very high affinity antibodies can have K_D in the picomolar (10^{-12}) range. While certainly a greater number of huMabs to LASV remain to be analyzed it is of interest that the three neutralizing huMabs analyzed to date can be considered very high affinity antibodies. A range of K_D values were observed for LASV GP huMabs that were not neutralizing, but most had average K_D values. LASV NP-specific huMabs also had average K_D values. Studies are in process to determine kinetic parameters of other LASV huMabs, both neutralizing and nonneutralizing. As values for binding kinetics can be subject to subtle changes in conditions, we will also confirm binding kinetics independently (Luis Branco, Zalgen).

Table 3. Binding kinetics of LASV huMAbs.

huMAb	Specificity	Neutralizing	ka (1/Ms)	kd (1/s)	KA (1/M)	KD (M)
19.7E	GP1-A	+	459	2.26E-08	2.03E+10	4.94E-11
10.4B	GP1A, GP2C	+	4.57E+03	1.16E-07	3.95E+10	2.53E-11
36.9F	GP2-A2	+	6.64E+04	9.08E-08	7.31E+11	1.37E-12
9.9F	GP1/BC	-	88.5	5.41E-05	1.64E+06	6.11E-07
20.5G	GP2-A or C	-	9.56	1.05E-05	9.08E+05	1.10E-06
22.5D	GP2-B	-	252	1.47E-03	1.72E+05	5.83E-06
38.2D	tbd	-	5.74E+03	2.80E-03	2.05E+06	4.87E-07
4.2F	tbd	-	5.78E+04	4.40E-07	1.32E+11	7.60E-12
1.1A	NP	NA	1.48E+04	6.74E-05	2.20E+08	4.55E-09
10.10G	NP	NA	2.03E+04	6.23E-04	3.25E+07	3.07E-08
10.5D	NP	NA	1.52E+04	8.35E-05	1.81E+08	5.51E-09
10.1C	NP	NA	1.84E+04	1.50E-04	1.23E+08	8.14E-09

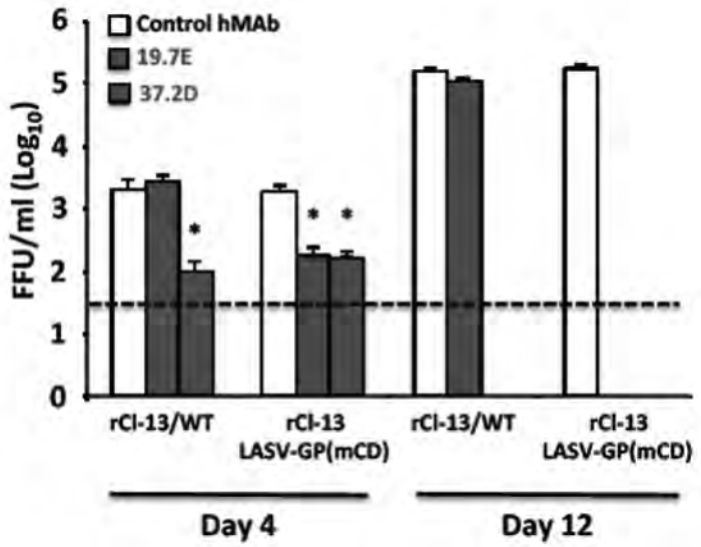


Figure 22. In vivo neutralization of LASV hMAbs 37.2D and 19.7E: B6 WT mice were treated with the indicated LASV GP-specific hMAb (20 mg/kg, i.p.; N=8/group for 19.7E and 37.2D hMAbs treated mice and N=4/group for control hMAb treated mice) and infected with rCl-13/WT or rCl-13/LASV-GP(mCD) (2 x106 pfu; i.v.). At 4 and 12 days post-infection, blood samples were collected and serum virus titers determined. Dotted line represents limited of detection (50 FFU/ml). * Virus was detected only in 3 out of 8 mice, whereas the other 5 mice in each group had viral titers below the limit of detection. *Data by Luis Martinez and Juan Carlos de la Torre.*

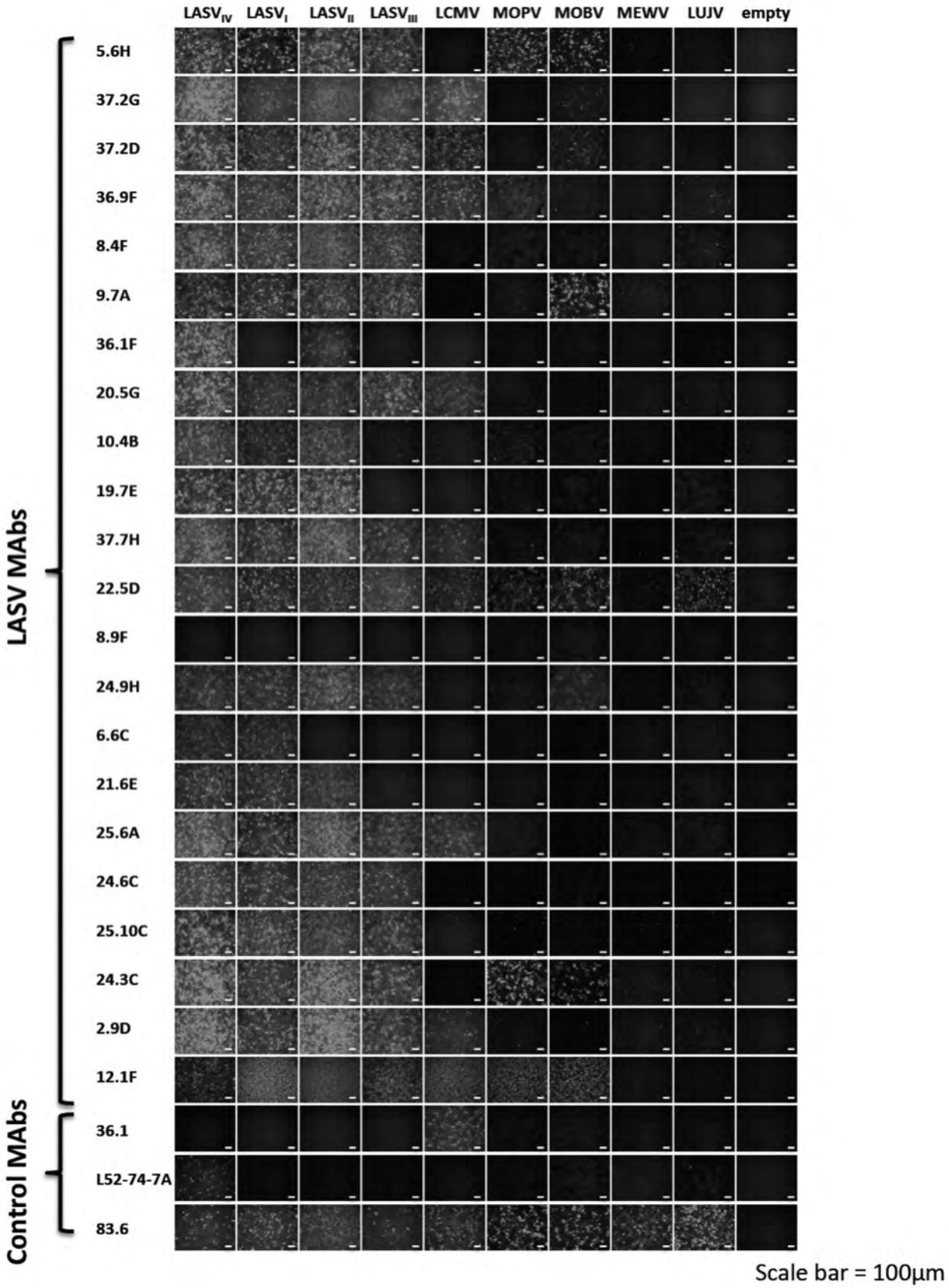


Fig. 23. Cross-reactivity of LASV GP-specific huMAbs with Old World arenaviruses. *Data by Luis Martinez and Juan Carlos de la Torre.*

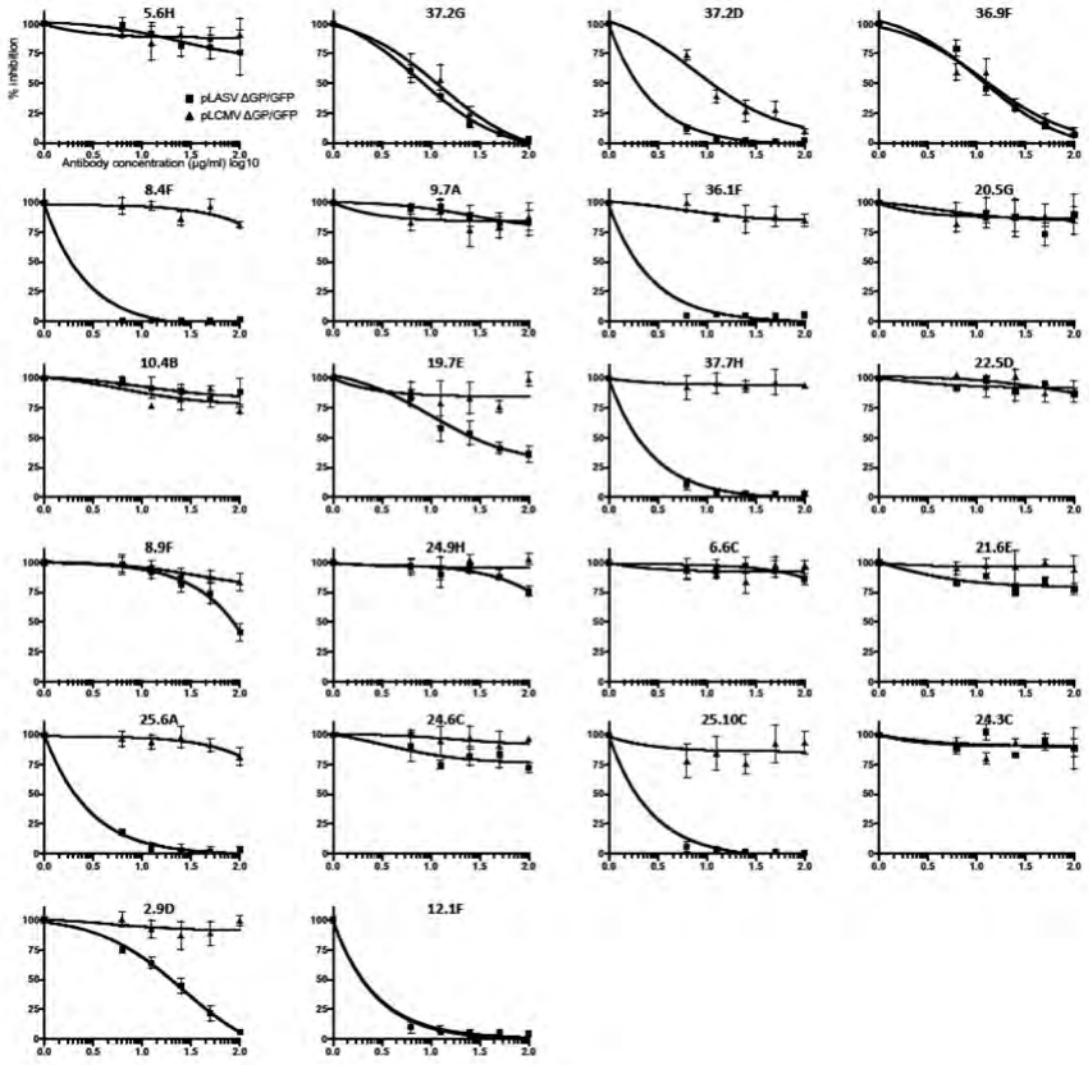


Fig. 24 . Neutralizing activity of LASV GP-specific hMAbs against LCMV. *Data by Luis Martinez and Juan Carlos de la Torre.*

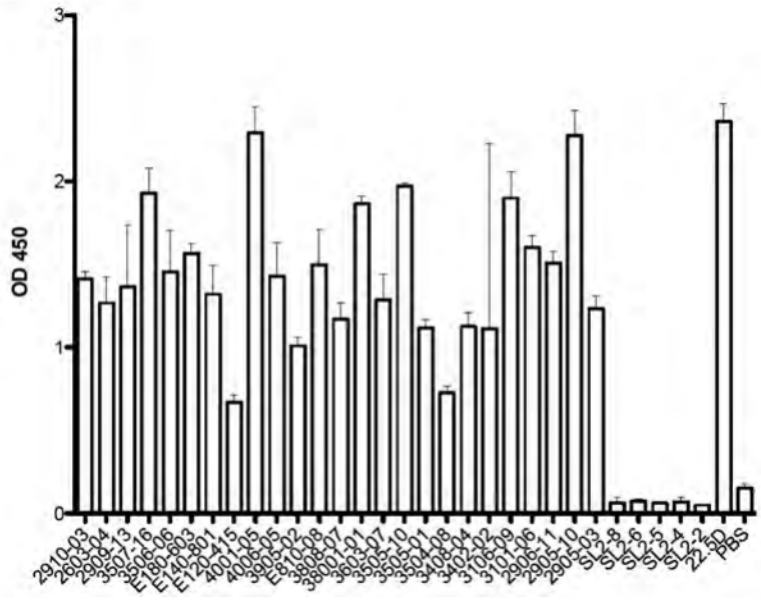


Fig. 25. Binding of serum from Lassa fever survivors to peptide 38, which represents the linear epitope recognized by huMAbs in group GP2-B. Bars labeled with SL are from seronegative subjects not exposed to LASV. 22.5D is a LASV huMab of group GP2-B that recognizes a linear epitope represented by peptide 38. PBS is a saline control. The remainer of the serum samples tested were from seropositive subjects that survived exposure to LASV.

Quantify reactivity of sera and MAbs to synthetic peptides.

As discussed above the only group of LASV GP-specific huMabs to recognize a linear epitope is GP2-B. Initial studies suggest that the epitope recognized by huMAbs of Group GP2-B is an immunodominant epitope recognized by most LF survivors (Fig. 25). Studies to determine whether or not the reactivity to this and other epitopes is a correlate of protection against fatal LF represent a high priority for the coming year's work.

Milestone 3: Determine X-ray crystallographic structures of the GP, NP and Z proteins of LASV and other arenaviruses in complex with human MAbs (The Scripps Research Institute-TSRI).

Goals:

- Scale-up LASV GP, NP and Z expression for crystallography.
- Data Collection and GP:MAb, NP:MAb and Z:MAb Structure Determination.

OVERVIEW OF CONTRACT DELIVERABLES ACHIEVED IN MILESTONE 3.
We fulfilled Milestone 3 contract goals.

- Scale-up of LASV GP, NP and Z expression for crystallography was achieved.
- Structure determination of arenavirus GP, NP and Z were all achieved. The crystal structure of LASV NP N-terminal domain, revealed RNA binding site and RNA gate. The crystal structure of LASV NP C-terminal domain, revealed 3'5'-exonuclease fold and immunsuppressive function in digestion of dsRNA and provides hypothesis for arenavirus RNP assembly and functional analysis. The crystal structure of LASV Z – oligomer suggests that the matrix protein is assembled via conformational rearrangement. The crystal structure of oligomeric LCMV GP: illustrates oligomeric association of a prefusion arenavirus GP and provided a template for mapping antibody epitopes and receptor-binding sites.

Arenavirus GP.
The arenavirus GP contains an N-terminal, transmembrane stable signal peptide (SSP), a GP1 subunit and a GP2 subunit, which bears C-terminal transmembrane and cytoplasmic domains. Crystal structures are available for the GP1 of Machupo virus, alone and in complex with transferrin receptor 1 [1, 13], the post-fusion six-helix bindle conformation of LCMV GP2 [14] and Guanarito GP2 [15], and the cytoplasmic domain of Junin virus [16]. No crystal structure is yet available for any prefusion, oligomeric GP1-GP2 complex. In this project, we have aimed to provide a crystal structure of oligomeric, prefusion arenavirus GP, and in particular, that of an Old World arenavirus so that antibody epitopes from survivors of Lassa infection could be mapped.

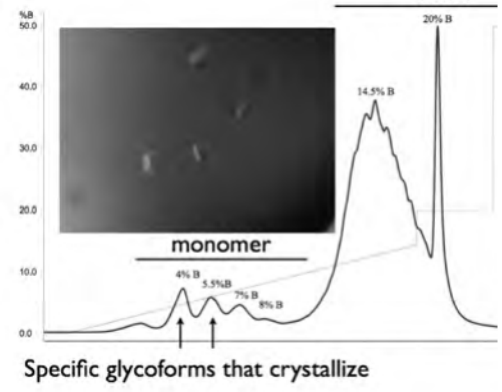


Fig. 26. Crystals of Lassa virus GP result from particular glycoforms. Engineering is in progress to generate a more homogenous population of these glycoforms and improve crystal diffraction.

Table 4. Selected anti-Lassa virus antibodies that cross-react to LCMV GP.

Antibody	Specificity
3.6H	GP2 - group GP2E
4.2F	GP2
6.7B	GP2
6.9A	GP2 – group GP2E
8.8B	GP2 – group GP2B
8.11C	pre-fusion GP2
9.7A	GP2 – group GP2A
10.4B	GP1/GP2 – group GP2C
10.5G	GP2 – group GP2D
13.4E	GP2
13.9H	GP2 – group GP2E
20.4F	GP2 – group GP2E
20.5G	GP2
21.5F	GP1 – group GP1B
22.2G	GP2
22.5D	GP2 – group GP2B
23.1D	GP2
23.2E	GP2
23.11A	GP2
34.8E	GP2
36.9F	GP2 – group GP2A
37.2D	GP2 – group GP2A
37.2G	GP2 – group GP2C
37.2H	GP2 – group GP2D
37.7H	GP2 – group GP2A
39.3G	GP2
A111	GP2
C132	GP1

The enzyme S1P cleaves in a flexible loop located between GP1 and GP2. Expression of soluble GP ectodomains bearing a wild-type S1P cleavage site often results in separation of the subunits from each other and purification of a GP1 fraction and a separate, “sprung”, post-fusion GP2 fraction. Mutation of the S1P cleavage site prevents cleavage, allows the GP1 and GP2 subunits to remain linked, and GP2 to remain in the expected prefusion conformation. For crystallization, we have screened wild-type, cleavable and also mutant, uncleavable forms of GPs.

Lassa GP: More than 100 versions of Lassa virus GP have been expressed in order to identify versions that are stable and crystallizable. GPs have been expressed in mammalian and insect cells, as cleavable and uncleavable forms, as GP1s and complete GPs, with native SSP or other leader sequences, and with a variety of truncations and point mutations to eliminate DXMS-mapped regions of flexibility. We now have weakly diffracting crystals of Lassa virus GP (Fig. 26), and find that they result from a minor fraction of the glycoprotein expressed: from protein missing one of the 11 possible glycosylation sites. We are now screening an array of Lassa virus GPs bearing point mutations to knock out this site alone and in combination with other glycosylation sites in order to improve diffraction of these crystals. We have also screened ~10 GP-single antibody complexes, but no crystals have resulted. However, we do have initial hits of two ternary complexes (GP + two separate Fabs). These crystals are currently being optimized. Two Fabs crystallized on their own from the single Fab-GP complex drops, with data collected to 3.4 Å and 1.8Å resolution, and structures determined. Hence, structures of these Fabs in their unbound forms are available if they would be helpful for paratope mapping or docking Fabs into envelopes of GP-Fab complexes measured by Small Angle Scattering or single particle electron microscopy. Efforts will continue in the next funding period to identify Fab-GP crystals, focusing on those Fabs that neutralize best and/or bind most stably with homogeneous stoichiometry as determined by single particle EM.

LCMV GP: We sought to crystallize other Old World arenavirus GPs in parallel, in case one would crystallize more easily than LASV and provide initial templates for understanding arenavirus biology and mapping antibody epitopes. Indeed, we have been able to determine a crystal structure of LCMV GP in its supposed oligomeric, prefusion conformation and present that crystal structure here.

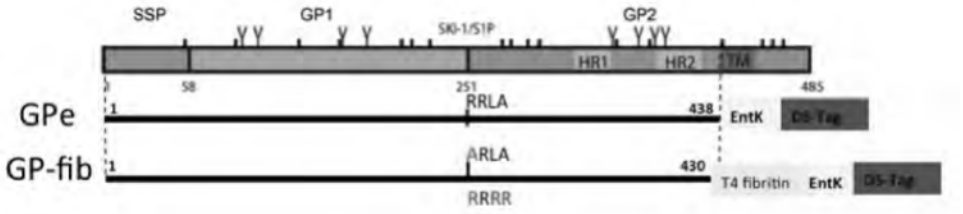


Fig. 27. At top is the organization of the wild-type LCMV GP. A soluble ectodomain (GPe) is made by deletion of transmembrane and cytoplasmic regions and addition of a removable double-Strep tag. A trimeric soluble ectodomain (GP-fib) is made by addition of a fibritin trimerization motif onto GPe. GP-fib has been made in uncleavable and furin-cleavable forms, bearing ARLA and RRRR sequences at the

Similarity to LASV GP: LCMV GPC and LASV GPC are 59% identical and 74% similar overall, and 50/67% identical/similar in GP1 and 71/85% identical/similar in the GP2 ectodomain. Notably, at least 28 antibodies identified in human survivors of Lassa virus infection cross-react to LCMV GPs produced for crystallization (Table 4).

Expression: LCMV GP was expressed in Drosophila S2 cells in several ways (Fig. 27). First, a soluble ectodomain was made that terminates immediately prior to the transmembrane segment. The ectodomain was linked via an enterokinase cleavage site to a double-Strep tag for purification. We call this construct GPe (ectodomain). This ectodomain was also expressed with a T4 fibritin trimerization motif at the GP2 C-terminus, followed by the enterokinase cleavage site and the strep tag. We call this construct GP-fib. All constructs were made in cleavable and uncleavable forms. GP-fib was further made as a furin-cleavable form, with an RRRR furin-cleavage site engineered between GP1 and GP2 for more homogenous and controlled cleavage. We call that GP-fib-furin. Proteins were secreted from S2 cells captured by Streptactin affinity and further purified by size exclusion chromatography coupled to multi-angle light scattering (SEC-MALS).

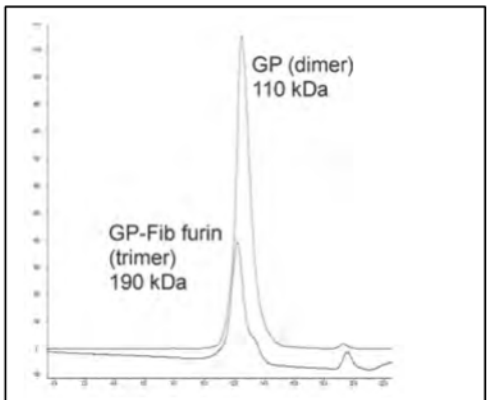


Fig. 28. SEC-MALS suggests that GPe forms a dimer, while GP-fib forms a trimer. The trimeric state of GP-fib remains after after the fibritin motif

Dimer: LCMV GP was expressed in *Drosophila* S2 cells as a soluble ectodomain that terminates immediately prior to the C-terminal, transmembrane segment of GP2. This construct, GPe (ectodomain), ends at a cleavable purification tag, and has no artificial oligomerization motif. Curiously, LCMV GPe yields stable dimers. The GP dimer resulted in our first diffraction-quality crystals. (Fig. 28). Dimers of GP have been observed before in analysis of protein-protein interactions observed in LCMV particles [17]. In our expression of recombinant ectodomains, we find the phenotype to be specific to LCMV: soluble ectodomains from LASV, Lujo virus, and Junin virus do not form dimer, but instead all form a mixture of monomer and trimer. Dimerization is likely mediated by the GP1 subunit of LCMV as GP1 expressed on its own also forms a dimer, although it exists in equilibrium with monomer, in the

absence of GP2 is also stably dimeric. The presence of the LCMV dimer is indeed curious, but the dimer grew our first crystals that diffracted well enough for structure determination (see below). Residues contained in the dimeric interface of LCMV GPs are not well-conserved among the arenaviruses, and thus this dimer structure may reflect a peculiarity of LCMV.

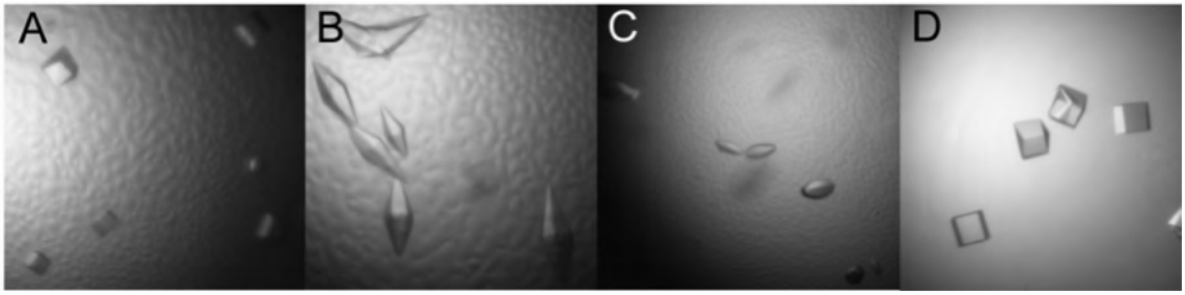


Fig. 29. **A)** Cubic crystals of GPe. **(B)** Octahedral crystals of GPe. **(C)** Ellipsoid crystals of GPe, with Strep tag removed. **(D)** Cubic crystals of GP-fib trimer.

Trimer: A stable population of only GP trimers can be generated by addition of a fibritin trimerization motif to C-terminus of GP2 in the ectodomain. These trimers were made in either uncleaved or cleaved forms (with GP1-GP2 linked or separated by enzymatic cleavage). This trimeric GP, GP-fib, remains stably trimeric after removal of the fibritin domain by trypsinization.

Crystallization: All proteins, dimeric and trimeric, cleaved and uncleaved, were screened for crystallization. All crystals were grown from fully glycosylated protein as deglycosylation, whether enzymatic or via point mutation, did not improve crystal or data quality.

The GPe dimers form crystals of three morphologies: cubic, elongated octahedral and ellipsoid (Fig. 29A-C). The cubic crystals diffract to 4-5 Å when frozen, but to 3.5 Å at room temperature. Crystals do not freeze well, are subject to radiation damage, and most diffract only weakly, if at all. Hundreds of native and derivatized crystals were screened at room temperature at multiple synchrotrons to obtain sufficient diffraction for structure determination. We ultimately collected native data to 3.5 Å as well as derivatized data to 4.5 Å for phasing and Sulfur-SAD data to 4.0 Å to confirm the polypeptide register. Electron density for seven out of nine potential N-linked glycans can be identified on each GP monomer in the final 3.5 Å maps. (Despite extensive optimization of cryo conditions and freezing techniques, data collected from crystals at room temperature was always superior.) A 3.5 Å native data set was generated by merging data from three crystals together. These crystals belong to the cubic space group I423 with unit cell dimensions a=b=c=263.3Å. The elongated octahedral crystals diffract to 7-8 Å, and belong to a hexagonal space group with unit cell dimensions a=b=91.8Å, c=250.0Å. The ellipsoid crystals result from enterokinase cleavage of the strep tag from the dimer and diffract to 9-10Å.

The asymmetric unit of the crystals contains two copies of a GP monomer. Together, they form a noncrystallographic, antiparallel dimer through interactions of both GP1 and GP2 subunits. One member of the dimer forms a trimer with neighboring copies of GP, centered about the crystallographic three-fold axis. The other member of the dimer makes no other oligomeric interactions. The GP trimer assembly is mediated by three sites of GP-GP interaction: (a) The N terminus of GP1 forming hydrophobic interactions to the neighboring monomer's fusion loop, (b) The C terminus of GP2 to the base of the neighboring monomer's fusion loop, and (c) a disordered loop of GP2 that extends into the center of the trimer axis.

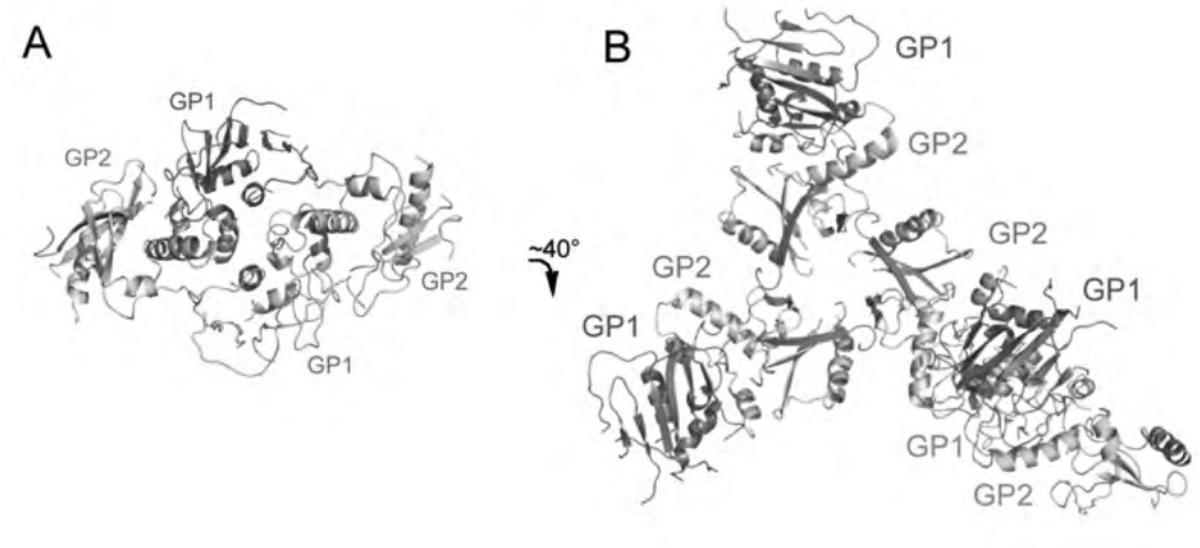


Fig. 30. Both dimeric and trimeric assemblies are observed in crystals. Illustrated is the current model: termini at center of trimer and loops throughout are still being built. **(A)** The asymmetric unit contains a dimer of GP. One monomer is colored with GP1 blue and GP2 green. The opposing monomer is colored entirely cyan. **(B)** The blue-green copy forms a trimer with neighboring copies of GP. The cyan GP does not. Each blue-green GP in the trimer interacts with one cyan GP. However, for clarity, only one of the cyan GPs is illustrated. In the trimer center, note the N-terminal strand of GP1 (blue) interacting with GP2 (green).

The GP-fib trimers, whether uncleaved or furin cleaved between GP1 and GP2, did not nucleate any crystals on their own or when seeded with crystals of GPe. We attempted limited proteolysis in order to find a version of trimeric GP that would crystallize, and found success with trypsin. Limited trypsinization of GP-fib removed the C-terminal membrane-proximal external region (MPER) of GP2, the fibritin trimerization motif and strep tag. Trypsin also cleaved the trimer at the S1P site between GP1 and GP2, after the RR in the RRLA motif (as determined by N-terminal sequencing). By size exclusion, the trypsinized protein remains as a single, trimeric species despite the proteolytic removal of the trimerization motif. Presumably, the trimerization motif encouraged assembly of a stable oligomer in producer cells, which remained stably oligomeric after its removal. The trypsinized trimer, when seeded by crystals of GPe, grows crystals of cubic morphology (Fig. 29D).

We have collected 4.0 Å from a single, frozen crystal of this GP trimer at SOLEIL beamline Proximus1. Although these crystals were grown from trimer, they belong to the same space group and have the same unit cell dimensions as crystals grown from dimeric GPe (I423, a=b=c=263.3Å). The identicality of the unit cell and space group suggests that the assemblies in the crystal are identical. (Note that the cubic space group has both 2- and 3-fold symmetry axes.)

Structure Determination: Crystals of GP dimer were obtained three years prior to crystals of GP trimer and a structure from crystals of dimeric GPe was pursued first. Structure determination was attempted by molecular replacement using the Machupo GP1 as a search model. Although multiple versions of the search model were attempted (complete GP1, alanine truncations, loop deletions, etc.), no molecular replacement solution could be identified. Regions of the postfusion six-helix bindle LCMV GP2 structure were also attempted as search models, but similarly yielded no solutions. After extensive screening of heavy atom compounds

and soaking conditions on crystals of dimer, screened at room temperature at SSRL beamlines 11-1 and 12-2, we identified a TaBr cluster that enabled structure determination by SIRAS. Heavy atom sites were identified using autoSHARP [18]. A Cα backbone trace was built using Buccaneer [19, 20], and the necessary rebuilding and refinement are in progress, using Coot [21] and Buster-TNT [22, 23], respectively. As the map is low resolution (3.5 Å), we wished to find additional sources of information to confirm the polypeptide register. Positions of the sulfur-containing Cys and Methionine residues in the model were confirmed via a Sulfur SAD experiment, using data collected to 3.75Å from a single, frozen crystal of dimeric GPe at SOLEIL Proxima 1. Further, electron density that resembles N-linked glycan can be identified at positions of the NXS/T motifs in the GP model.

The structure of a GP trimer was determined using the 4 Å native data collected from crystals of trimer, using a monomer from the rebuilt GPe dimer as a molecular replacement search model. Rebuilding and refinement of the trimer are also in progress, using Coot [21] and Buster-TNT [22, 23].

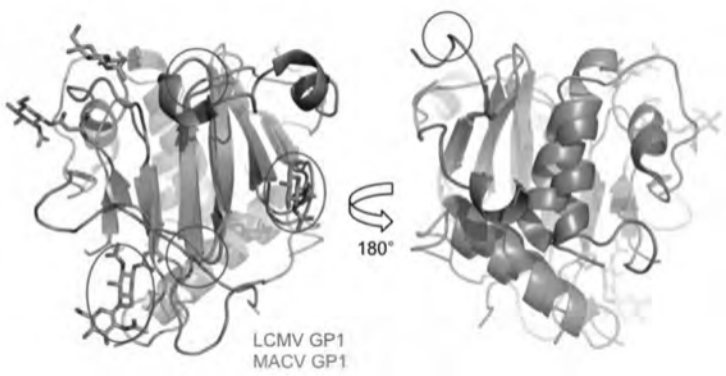


Fig. 31. Superimposition of MACV GP1 (orange,) and LCMV GP1 (blue). The central b sheet and two major helices are conserved. Connecting loop structures differ. MACV GP1 has four N-linked glycosylation sites. Glycans are illustrated in green ball-and-stick. LCMV GP1 has five sites, noted with red circles. Glycans have not yet been built but electron density is visible. Two sites are conserved with MACV.

Oligomeric organization: As one might expect from the identicality of the unit cells, crystals grown form dimer and crystals grown from trimer exhibit essentially identical crystal packing, and the copies of GP form both dimeric and trimeric interactions in the crystals.

The asymmetric unit of both crystals contains two copies of the GP monomer. The two copies in the asymmetric unit form a noncrystallographic, antiparallel dimer, mediated by both GP1 and GP2 subunits. One of the two monomers in this dimer makes no other oligomeric interactions. The other copy simultaneously assembles a trimer with neighboring copies of GP. The GP trimer is formed about a crystallographic 3-fold axis, and hence every monomer in it is structurally identical.

The dimer interface is extensive (Fig. 30A) and buries ~2150 Å of molecular surface, including GP1 residues 131-142 and GP2 residues 336-359. The biological relevance of this dimer is unknown. The arenavirus is expected to be a type I viral glycoprotein and to form a trimer on the viral surface. However, the extensive buried surface of the GP1-GP2 interaction is much more than one would typically expect for an artifactual interaction. We do note a cross-reactive neutralizing antibody from a Lassa virus survivor, mAb 19.5A, that is specific for the dimeric form of LCMV GPe. It is possible that this assembly might actually exist at some point in the virus life cycle. Type II viral glycoproteins, such as those of the flaviviruses, are known to adopt trimer-to-dimer and dimer-to-trimer rearrangements in maturation and fusion. One could certainly attempt to invoke a scenario in which dimers might appear on the LCMV surface

during the rearrangements of GP during fusion, perhaps driven by the ~40 amino acid cytoplasmic domains of GP which interact with matrix underneath. We note, however, that the two GPs in the asymmetric unit interact in an antiparallel fashion, so that in prefusion GP conformations, one would be oriented transmembrane-up while the other is oriented transmembrane-down. Hence, either this arrangement is either purely artifactual, occurs after GP1 is freed from GP2 during fusion, or occurs if GP subunits twist sideways during the rearrangements of fusion, instead of projecting purely "upwards". The relevance of this dimeric interface is unknown, but should be explored in the next funding period by mutagenesis and functional analysis of dimer-specific and trimer-specific neutralizing antibodies.

Trimer: We expect that the trimeric assembly of GPe observed in both types of crystals (Fig. 30B) is the assembly that is relevant for the prefusion GPC envelope spike, and the structure most relevant for neutralizing antibodies. Indeed, multiple cross-reactive neutralizing antibodies from Lassa virus survivors exhibit preferential binding of LCMV trimer over dimer (mAbs 3.6H, 6.9A, 13.9H, 21.5F, 22.2G, 23.1D, 23.2E, 34.8E, A111, and C132). This trimer arrangement is mediated by three sites. (a) The N-terminus of GP1 forms a loop, which makes hydrophobic contacts with the fusion loop of the neighboring monomer. (b) The C terminus of HR2 in GP2 makes contacts with the base of a neighboring monomer's fusion loop. (c) A third site of intersection is likely to occur involving a loop, which is not yet built, that extends from this GP2 β sheet into the center of the trimer axis. The unmodeled electron density in this region appears to be made by polypeptide of each of the three monomers in contact.

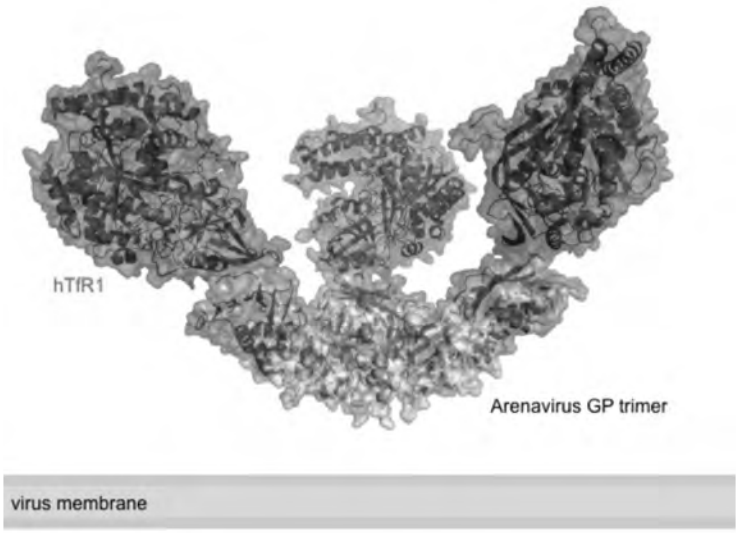


Fig. 32. Superimposition of the MACV GP1-TfR1 complex [1] onto LCMV GP1 in the LCMV trimer. TfR1 is colored magenta, superimposed MACV GP1 blue, and LCMV GP2 green.

The three copies of heptad repeat 2 (HR2, the C terminal helix) in GP2 do not form a coiled-coil in this trimer, but instead point inward toward the trimer axis. The 20-residue MPER region C-terminal of HR2 is attached, but disordered in crystals grown from dimer. In crystals of trimer, it has been removed by trypsin. The location of the MPER attachment point immediately at the base of the observed trimer suggests that it forms the stalk region of the trimeric spike.

We expect that the strongest anchors of trimerization of intact viral-surface GP are the regions that are not contained in the crystal structure: the trio of transmembrane subunits on GP2, as well as the six additional transmembrane subunits contributed by the three copies of SSP.

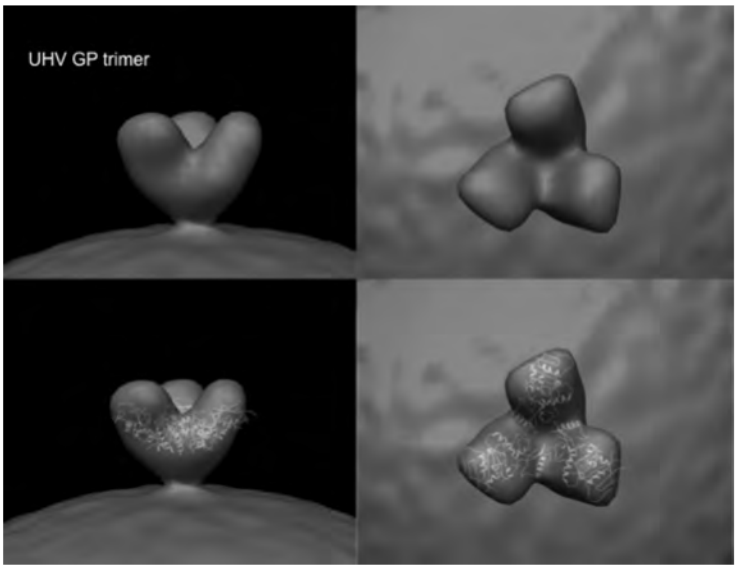


Fig. 33. Top, cryoEM reconstructions of GP spikes from the University of Helsinki arenavirus (UHV) [3]. Bottom, LCMV trimer (yellow) modeled into spikes.

S1P Cleavage site: Residues that comprise the S1P cleavage site lie in a 22-residue loop that is disordered in the crystal structure, and that, in DXMS, exhibits the most rapid solvent exchange of any portion of GPe. The cleavage site is probably quite flexible. In this loop, an F260L mutation, which occurs in the Armstrong53 strain of LCMV has been implicated in conferring lower receptor affinity and an acute, as opposed to persistent, course of infection [24-26]. It is currently unclear of the F260L alters a direct contact of α-dystroglycan, the likely receptor for Old World arenaviruses [27-29], or if this mutation has a longer-range structural effect on receptor binding. Once the crystal structure is completely built and refined, we will use it to choose a series of point mutations in order to map the binding site of α-dystroglycan. This work will be performed in the next funding period, and corresponding mutations will be made in both LCMV and LASV.

Comparison to Machupo virus GP1: Machupo virus (MACV), belongs to the New World clade of arenaviruses, and uses the protein Transferrin receptor 1 (TfR1) as its receptor. Crystal structures are available for both MACV GP1 [13] and MACV in complex with TfR1 [1]. Overall, LCMV and MACV GP1 share a common fold, including the central β sheet and two helices (Fig. 31). Superimposition of MACV GP1 onto LCMV reveals that MACV GP1 fits very well into this LCMV trimer structure with no clashes of polypeptide or glycan with GP2 or neighboring monomers. In this arrangement, the TfR1 binding site of MACV would be on the upper face of each GP1 in the trimer (Fig. 32). TfR1 appears to bind on the opposite face of GP1 than the S1P cleavage site.

Comparison viral surface spikes: It seems possible, perhaps likely, that Old World and New World arenaviruses adopt the same trimeric arrangement. The LCMV trimer indeed fits into cryoEM reconstructions of the trimeric envelope spikes of the University of Helsinki arenavirus (UHV), currently the only cryoEM reconstruction of GP on an arenavirus surface. In the top view (Fig. 3, right) the LCMV GP trimer (yellow) appears to fit the shape and spacing of the virus-surface trimer well. In the side view (Fig. 33, left), additional density is visible above and below the crystal structure. The density above could represent the large amount of O-linked glycan that is particular to UHV and absent in LCMV, or a somewhat different angle of the trimer, or the five as-yet-unmodeled N-linked glycans attached to LCMV GP1. The density

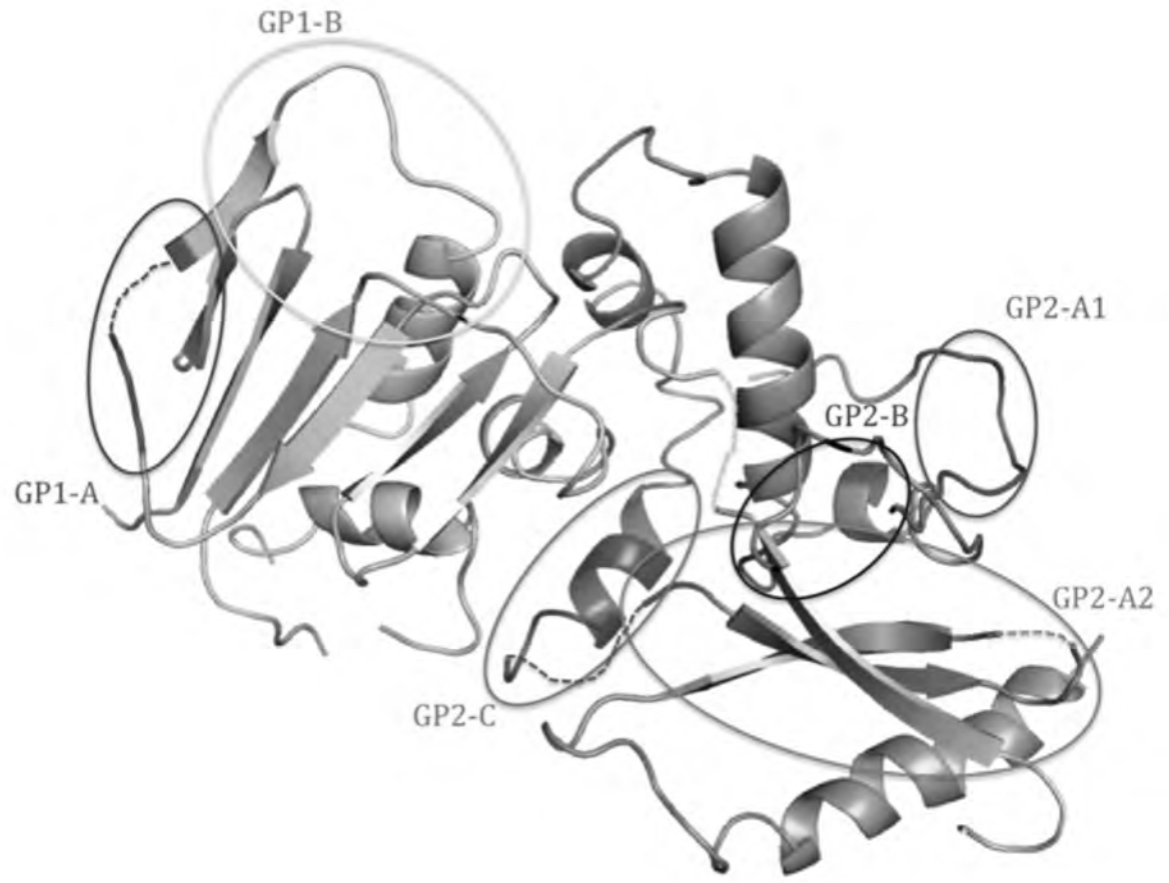


Fig. 34. Antibody epitopes mapped onto the GP monomer. GP1, outside likely epitopes, is colored light blue. GP2, outside key epitopes, is colored green. In GP1 are groups GP1-A, which includes the neutralizing antibody 19.7E, and GP1-B/C, which includes non-neutralizing antibodies such as 19.5A and 2.4F; and GP2-A1 (pink), GP2-A2 (orange, and also includes a beta-strand from the extreme N-terminus of GP1), GP2-B (blue), GP2-C (purple), and GP2-D/E (not shown). All neutralizing antibodies recognizing GP2 epitopes fall into epitope classes GP2-A1/A2 and GP2-C.

below could belong to the MPER stalk region missing from the crystal structures. The fit of the LCMV trimer, however to this very disparate arenavirus, suggests that the oligomeric arrangement observed in the LCMV crystals may be shared across the arenavirus family.

Where do antibodies bind on GP? The main goal of this program is to map B cell epitopes on the arenavirus GP. Although the structure is still in refinement, we indeed can begin to map key epitopes and competition groups (Fig. 34). Antibody epitopes can, thus far, be roughly classified into seven different competition groups. In GP1 are groups GP1-A, which includes the neutralizing antibody 19.7E, and GP1-B/C, which includes non-neutralizing antibodies such as 19.5A and 2.4F. In GP2 are groups GP2-A1 (pink), GP2-A2 (orange, which also includes a β–strand from the extreme N-terminus of GP1, GP2-B (blue), GP2-C (purple), and GP2-D/E (not shown). All neutralizing antibodies recognizing GP2 epitopes fall into epitope classes GP2-A1/A2 and GP2-C. Epitope napping, using the fully refined structure, as well as collaborative mutagenesis and DXMS, will continue in the next funding period.

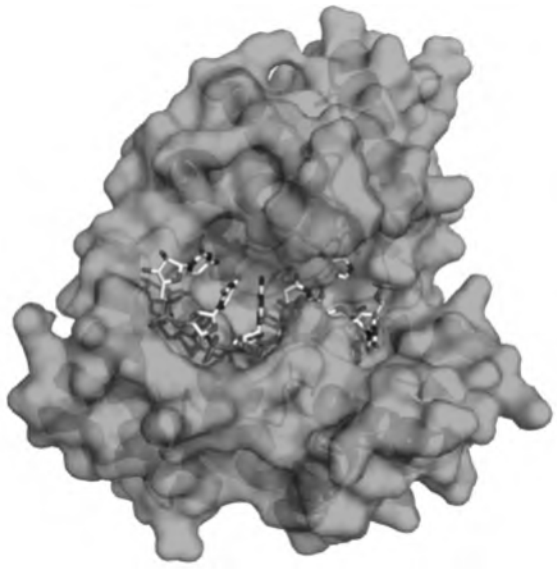


Fig. 35. ssRNA (ball and stick) tunnels through a crevice in the N-terminal domain of LASV NP (green).

The Nucleoprotein - NP

NP is the most abundantly produced protein during viral infection and it is multifunctional, playing key roles in host immunosuppression, viral replication, and encapsidation of the viral genome. NP is divided into N- and C-terminal domains. The C-terminal domain is immunosuppressive. Our crystal structure and accompanying functional analysis revealed the C-terminal domain to be folded and function as a 3'-5' exoribonuclease that specifically and rapidly digests double-stranded RNA (dsRNA) [30, 31]. Our crystal structure of the N-terminal domain revealed RNA bound into a specific crevice tunneling through the N-terminal domain [32] (Fig. 35).

The oligomeric assembly of NP and the bound genomic RNA, within the ribonucleoprotein complex remains unknown. There is one crystal structure of full-length LASV NP [33]. In this structure, NP crystallized as a trimer, but the trimeric interface maintains NP in a conformation unable to bind RNA [34]. Some conformational change, rearrangement, or different assembly of NP must occur in the actual RNP.

In an effort to further our understanding of the structure and function of the arenavirus nucleoprotein, we determined the X-ray crystal structure of the C-terminal domain of LCMV NP (termed NPΔ340). We obtained crystals in both $P2_1$ and $P6_5$ space groups, which diffracted to 2.8 and 2.0 Å, respectively. Data were indexed using d*TREK [35], structures were determined by molecular replacement, built in *Coot* [21], and refined with Phenix [36]. As expected, LCMV NPΔ340 adopts the same exonuclease fold with an r.m.s.d. of 0.49 Å between the backbones of the LCMV and LASV structures, and coordinates the same structural Zn to the residues E392, C499, H502, and C518 and a catalytic Mg ion to residues of the DEDDh exonuclease motif (D382, E384, D459, D522, and H517).

An interesting NP-NP interaction, observed in both $P2_1$ and $P6_5$ structures of LCMV and all NPΔ340 structures of LASV, involves insertion of the ultimate C-terminal residue of LCMV NP into a hydrophobic pocket of a neighboring NP monomer. Both the C-terminus and the recipient hydrophobic pocket are largely conserved across arenaviruses. The packing of the P65 crystals involves an additional type of interaction: a protein-protein interface centered about D471, in which D471 forms a salt bridge to R469 of the neighboring monomer. Previous functional studies have found that residue D471 of LCMV NP is essential for oligomerization and replication, but why it is essential is not known [37].

The D471 crystal-packing interface, combined with the C-terminal interaction described above, results in the formation of a left-handed double helical assembly of NPs in the crystals (Fig. 36A,B). Could one of the two helical strands represent an interaction observed in the RNP? This crystal structure is of only the C-terminal domain of NP, and of course, it is the full-length protein that assembles the RNP. Alignment of the full-length LASV NP structure (PDB ID:

3MWP) [33] to the LCMV C-term domain demonstrates that each single helix formed by C-terminal domains easily accommodates the N-terminal domains if attached (Fig. 30C). The full-length NP structure is RNA-free. Superimposition of the RNA-bound N-terminal domain onto

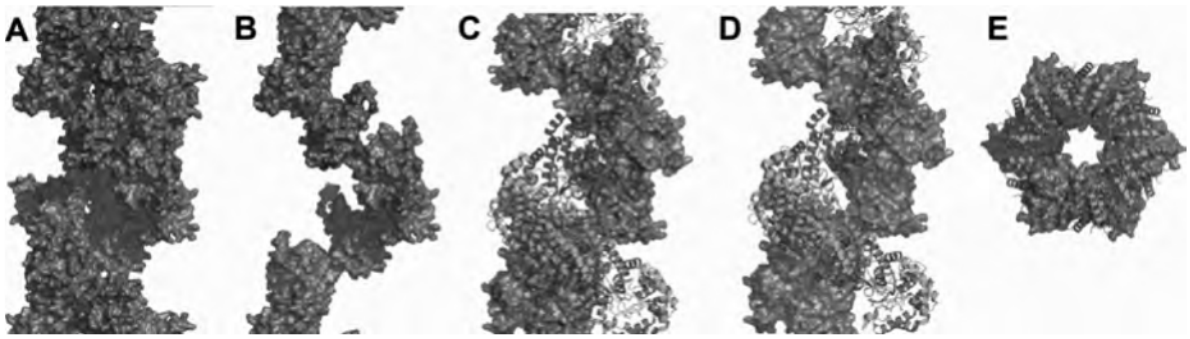


Fig. 36. **A)** Double-helical filaments formed in $P6_5$ crystals by two chains of LCMV NP C-terminal domains (blue). **(B)** One single helical assembly formed by one of the two chains of the double helix in (A). **(C)** Superimposition of the full-length LASV NP onto the LCMV NP C-terminal domain arrangement in (B). The LASV N-terminal domain is illustrated in green ribbon. The LASV C-terminal domain is illustrated in blue molecular surface. **(D)** Superimposition of the LASV N-terminal domain–RNA complex onto the LASV NP shown in (C). The ssRNA oligos (8 nucleotides each) are illustrated in a red thread. **(E)** Top view of the helical assembly of (D). The N-terminal domain of LASV NP is in green. The C-terminal domain is in blue and RNA in red.

the N-terminal domain of the full-length NP in this helical filament (Fig. 30D) illustrates that the 8-nucleotide bound RNA segments are all oriented with the 5' at one end of the helix and the 3' end toward the other. Interconnecting RNA genome that would link the tightly bound segments could pass between N- and C-terminal domains to form one single length of ssRNA genome coiling about the NPs.

In this helical filament there are six monomers per turn, with a pitch of 146 Å and a diameter of 113 Å. The clearest images of an arenavirus RNP are those of the New World arenavirus Pichinde (50% identical in NP sequence to Old World arenavirus LCMV). The Pichinde arenavirus RNP is observed to form a helical filament 120-150 Å in diameter in physiological ionic strength, that partially disassembles into a beads-on-a-string morphology when dialyzed into low ionic strength [38]. The difference in diameter of the LCMV model and the Pichinde EM could be the result of experimental error, expansion of the natural NP helix upon binding of RNA or inherent differences between Old World and New World viruses. In the next funding period, we would like to explore this model by mutagenesis of the assembly interfaces and analysis of the resulting NP interactions, replication capacity and RNP assembly.

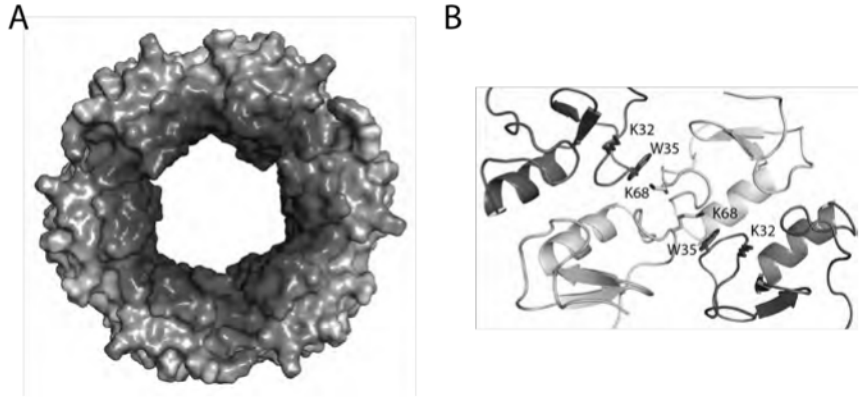


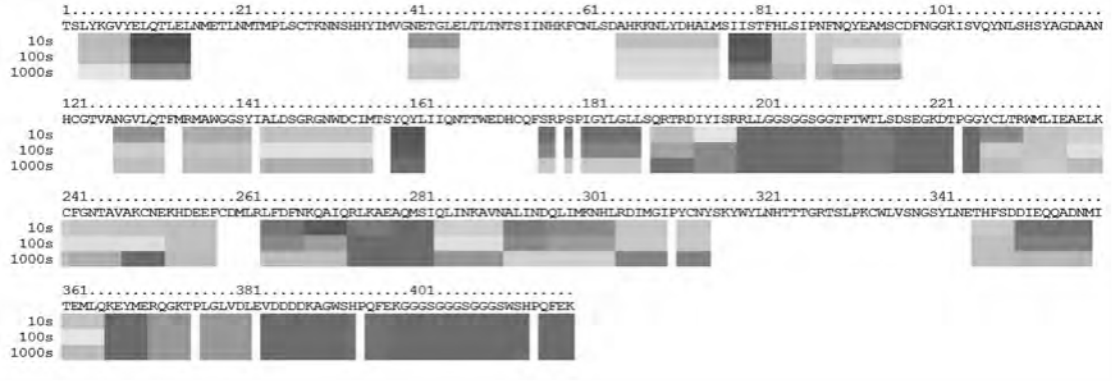
Fig. 37. Structure of the oligomeric form of LASV Z. A) Electrostatic surface potential of oligomeric Z shows the center of the wreath is highly basic. Positive surface is colored blue; negative surface is colored red with limits ± 10 kT/e.b. (B) The underlying structure of the basic inner surface of the wreath. Residues Lys32, Trp35 and Lys68 contribute to the basicity.

LASV Zinc binding (matrix) protein Z

The matrix protein Z of the arenaviruses occurs in monomeric and oligomeric forms, is necessary and sufficient for viral assembly and budding, negatively regulates viral replication and transcription and is important for the suppression of both viral and host cell translation. We have determined the crystal structure of the dodecameric form of Z from Lassa virus to 2.9Å (Fig. 37). The Z dodecamer forms in a concentration and time dependent manner, is SDS-resistant and is comprised of six dimers of Z that come together to form a donut-shaped ring. Notably, the interior of this ring is highly basic, as it is lined with two copies each of residues K32 and K68 that sandwich two copies of W35. Mutations of K32 and W35 to alanine and K68 to glutamate greatly reduce the ability of Z to inhibit replication and transcription of a Lassa virus mini-genome. Whether the reduction in inhibition is due to a change in the propensity to oligomerize (for example, K32A forms dodecamers faster and at a lower concentration than wild-type Z) or is due to a direct interaction of these residues with the replication machinery (L and NP) has yet to be determined.

Further, it is as yet unclear if this ring is a building block of the assembly required for budding. Wild-type Z is released into the supernatant of transfected cells in the form of budded, Z-containing virus-like particles. However, the Z mutant L56R, which is unable to form the dodecamer, is also released into the supernatant. In contrast, mutations to residues not blocking ring formation *are* disruptive to budding (i.e. K32E, W35A, K68E). It might be that budding of virus-like particles is simply robust, or that a different assembly is involved in budding, although no other assembly is noted from purified Z.

Ribbon Map of LasGPMPER5 Monomer alone (KHES_65)(in D%, Deuteration Level)



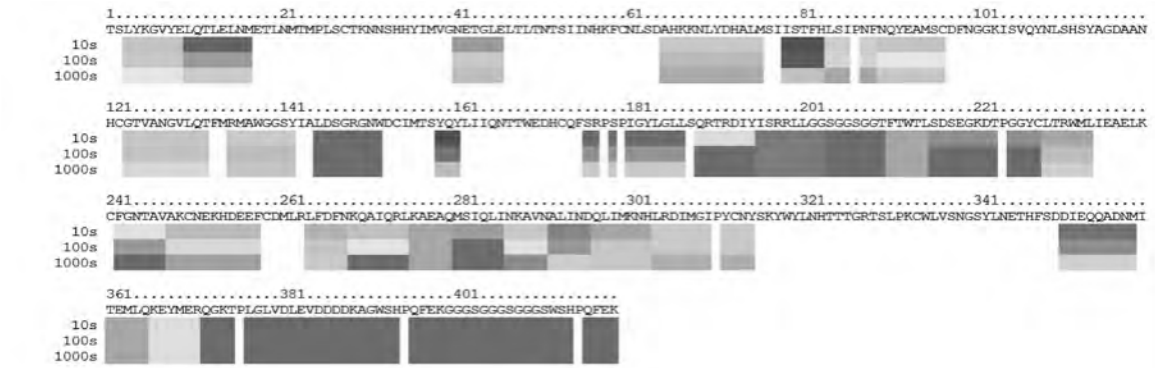
Ribbon Map of LasGPMER5 in LasGP:10.4B Complex (KHES_66) (in D%, Deuteration Level)

Influence of 10.4B (in D%, Deuteration Level)

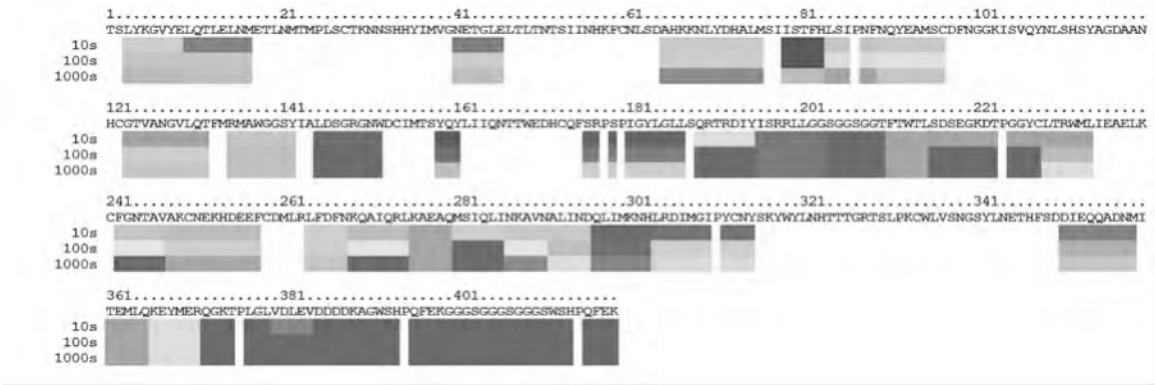
Blue suggests the regions which exchange slower upon 10.4B binding; And red suggests the regions which exchange faster upon binding.

Fig 38. DXMS studies to identify epitopes in 10.4B, a huMAb of group GP2-C Upper Panel; Deuteration of LASV GPC in the absence of antibody. Middle Panel: Deuteration of LASV GPC in the presence of antibody. Lower Panel: Map showing differential binding in the T loop region of GP2.

Ribbon Map of LasGPMPER5 Monomer alone (KHES_69)(in D%, Deuteration Level)



Ribbon Map of LasGPMER5 in LasGP:37.2G Complex (KHES_71) (in D%, Deuteration Level)



Influence of 37.2G (in D%, Deuteration Level)

Fig 39. DXMS studies to identify epitopes in 37.2g, a huMAb of group GP2-C. Upper Panel; Deuteration of LASV GPC in the absence of antibody. Middle Panel: Deuteration of LASV GPC in the presence of antibody. Lower Panel: Map showing differential binding in the T loop region of GP2.

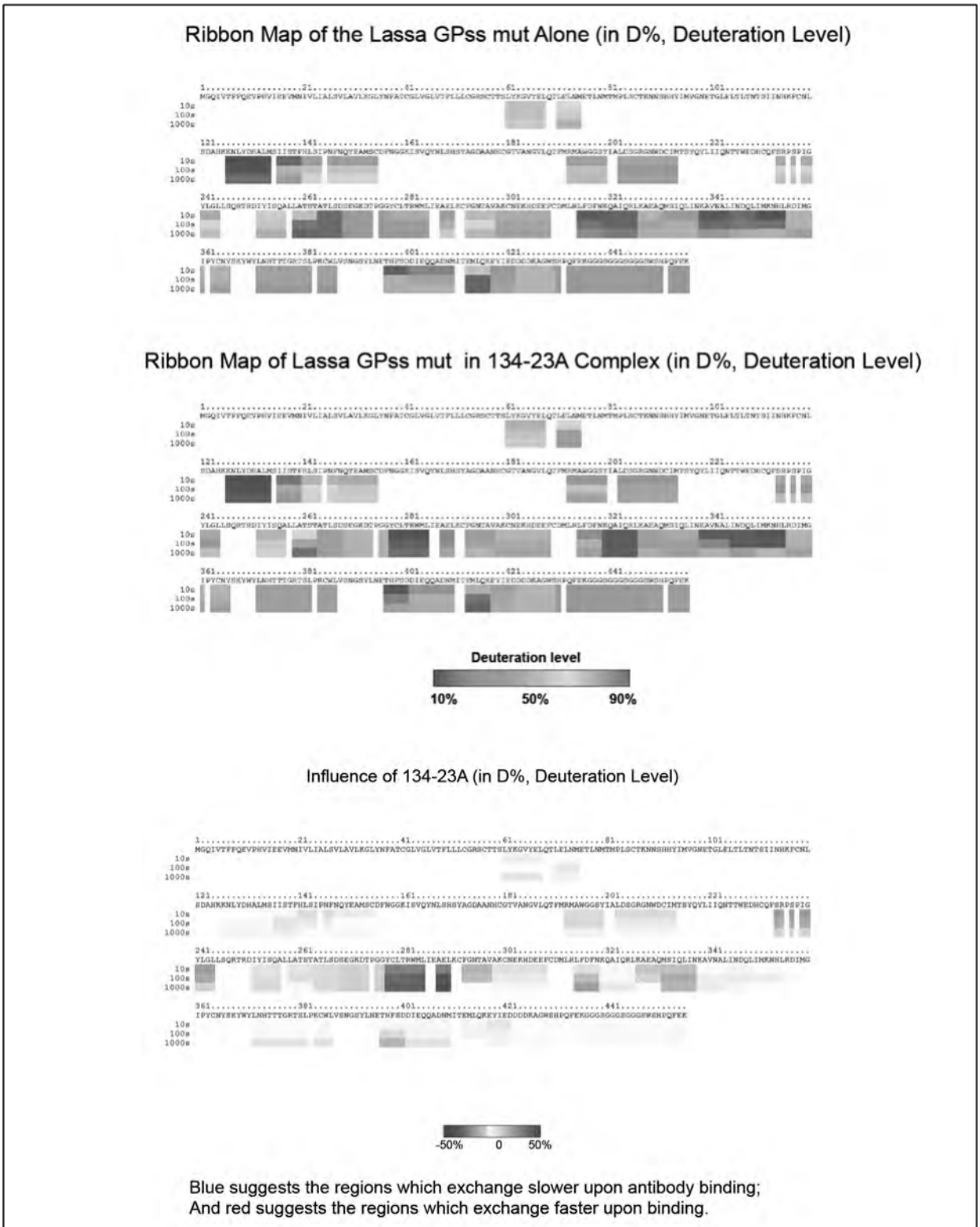


Fig 40. DXMS studies to identify epitopes in a mouse LASV GP2 monoclonal antibody. Upper Panel; Deuteration of LASV GPC in the absence of antibody. Middle Panel: Deuteration of LASV GPC in the presence of antibody. Lower Panel: Map showing differential binding in the fusion peptide region of GP2.

Milestone 4. Develop novel screening methods for LASV B cell epitope identification, including deuterium exchange mass spectrometry and small angle X-ray scattering (TSRI and UCSD).

Goals:

- Perform DXMS analysis of 100 LASV GP:MAb complexes, 50 LASV NP:MAb complexes and 25 LASV Z:MAb complexes.

OVERVIEW OF CONTRACT DELIVERABLES ACHIEVED IN MILESTONE 4.
We will fulfill modified Milestone 4 contract goals.
We developed DXMS as a tool to identify linear and conformational epitopes. DXMS is now widely used by other B cell epitope groups. As directed by the epitope advisory group our focus has been on LASV GP.

DXMS is able to describe solvent exposure and mobility of each region of a protein or protein complex by employing peptide amide hydrogens as water-accessibility probes. One amide hydrogen is present on each amino acid in a protein except proline. Each peptide amide hydrogen continuously and reversibly interchanges with hydrogen present in water, in a manner reflecting the solvent accessibility and thermodynamic stability of the peptide bond to which it is attached. By allowing these hydrogens, within the structured protein, to exchange with solvent deuterium (as D_2O), acid pH-quenching the reaction (greatly slowing further exchange), digesting the protein with pepsin, and analyzing the resulting deuterium-exchanged peptides by mass spectrometry, we can determine which amino acids of a protein are exposed to solvent and which are buried. DXMS has been established as a method to map conformational (discontinous) and linear epitopes on complex proteins. After the untimely passing of UC San Diego subcontract Director Dr. Virgil L Woods, Jr., Dr. Wood's long-time associate Dr. Sheng Li took over running the UCSD DXMS Proteomics Resource.

DXMS LASV GP
Deuterium-exchange mass spectrometry (DXMS) maps have been produced for LASV GP, Z and both the N- and C-term domains of LASV NP. LASV GP2-C antibodies 37.G and 10.4B are mapped to LASB GP amino acids 350-360 based on DXMS data (Figs. 38, 39). As noted above, the mapping of 10.4B by DXMS conflicts with other results including mutagenesis data that place it in group GP1-A (Fig. 4). Given that 10.4B has shown that ability to partially protect guinea pigs from fatal LF we intend to resolve these discrepant results, which we believe will be revealing. DXMS Mapping of a mouse antiLASV GP MAb by Dr. Li, revealed that the antibody binds to the fusion peptide region of LASV GP2 (Fig. 40).

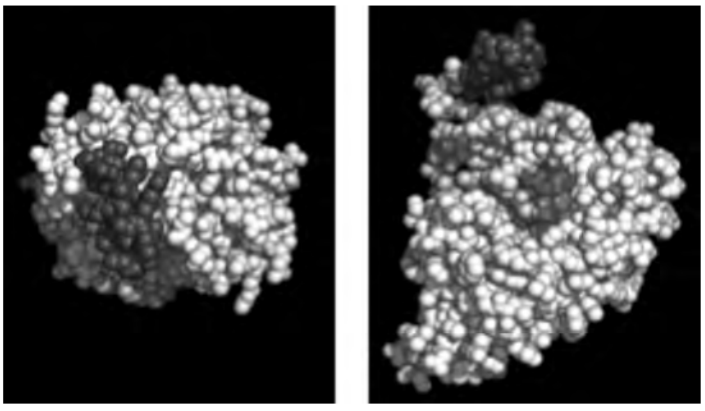


Fig. 41. DXMS analysis of binding of NP MAb 1474. Two views of NP. Solvent exposed molecules that become solvent protected after Ab binding are shown in red.

DXMS LASV NP
DXMS-mediated epitope mapping of Lassa NP with a panel of antiNP mAbs is underway, and our first results with the mAb1474 have just been completed (VLW lab). All amino acids in the NP protein construct studied (residues 1-340) were assessed in this comprehensive study. Remarkably, each of the fourteen amino acids constituting the linear sequence 130-143 of NP contribute to the

binding epitope for the antibody, with no evidence of binding or induced conformational change outside this single contiguous region (Fig. 41). DXMS analysis indicates that while this entire region is very solvent-exposed before antibody binding, it becomes highly protected upon antibody binding. The crystal structure of NP shows this region to be a flexible helix connected with other parts of the protein by very flexible loops.

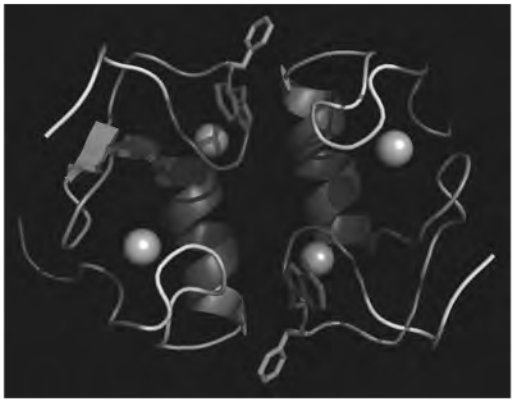


Fig. 42. 10 second on-exchange DXMS map of the LASV Z dodecamer mapped onto the structure of the repeating dimeric building block. On exchange rates are shown as a heat map, ramped from the slow-exchanging residues in blue to the faster-exchanging residues in yellow. Trp 35 is blue (slow exchanging) while Phe 36 is green (medium exchanging).

DXMS LASV Z

DXMS analysis of LASV Z suggests that Trp35, which has been shown to be important for interaction with the cellular translation initiation factor eIF4E, appears to be stably buried in the dodecamer interface of LASV Z (Fig. 42). The alpha helix, which stabilizes the dimer interface, as well as the residues surrounding the Zn atoms are also stable. Residues corresponding to the N- and C-terminal portions of LASV Z are not seen in our crystal structure and are found to be very fast exchanging by DXMS, indicating these portions of Z are highly flexible.

B. EPITOPE VALIDATION

Milestone 5: Define the kinetics of IgM- and IgG-responses to defined epitopes on GP, NP and Z proteins of LASV using variations of LF ELISA and ELISPOT assays in acutely-infected, convalescent, and previously-exposed persons (Tulane).

Goals:

- Qualitative, quantitative and temporal assessment of IgG and IgM reactivity to LASV proteins in 50-75 LF patients per Quarter.
- Quantify LASV specific B cells in 20 LF patients per Quarter.
- Define immunodominance patterns to specific B epitopes in 50 LF patients per Quarter.

Define responses to specific epitopes in 100 LF patients per Quarter

OVERVIEW OF CONTRACT DELIVERABLES ACHIEVED IN MILESTONE 5.
We will fulfill modified Milestone 5 contract goals.

- Qualitative, quantitative and temporal assessment of IgG and IgM reactivity to LASV proteins was conducted for 50-75 LF patients per Quarter.
- We found that LASV specific B cells overwhelming were reactive to LASV NP, rather than GP. Because the epitope advisory committee stringly indicated that we should foicus on LASV GP, we did not pursue this goal further .
- We will continue to assess immunodominance patterns to specific B epitopes and to define responses to specific epitopes in LF patients as a high priority.

We met our goals qualitative, quantitative and temporal assessment of IgG and IgM reactivity. As previously described, the Lassa B cell epitopes program is defining a important, previously unknown, aspect of LASV immunopathogenesis. IgM titers persist in Lassa fever survivors for extended period of time up to years (Fig. 44). The early IgG response to the GP and Z proteins is suppressed even in Lassa ever survivors. Typically titers IgG antibodies to the GPs and Z are low or absence at time of discharge (not shown) those eventually these antibodies develop. There are several potential mechanisms for prolonged LASV-specific IgM titers: 1) A prolonged and as of yet largely uncharacterized inhibition of class switching could be prevalent in LASV infections; 2) Sustained IgM titers could be generated by IgM+ memory B cells; 3) Impaired CD4+ T helper lymphocyte function during LASV infection, with long-term impact on class switching.

Evidence for these interpretations can be found in the literature. Non-neutralizing virus-specific IgM were analyzed and deemed crucial for impedance of viral persistence in LCMV infection (the prototypic Old World arenavirus), suggesting that IgM-producing cells have remained largely obscure and underappreciated in the characterization of arenaviral infections. The late appearance of neutralizing antibodies in arenaviral infections has been tied to high viral antigen-to-B cell ratios and low T cell help, which resulted in a normal IgM response but reduced the efficiency of class switching (Fig. 43). [39, 40] Lowering the antigen-to-B cell ratio and increasing T cell help resulted in rescuing of class switching and emergence of neutralizing IgG specificities. Thus it is possible that a normal early IgM response in LASV infections is followed by an impaired T helper cell response with sustained IgM production and maturation of the producing B cell subset into an IgM+ B cell memory population. We are actively testing these hypotheses.

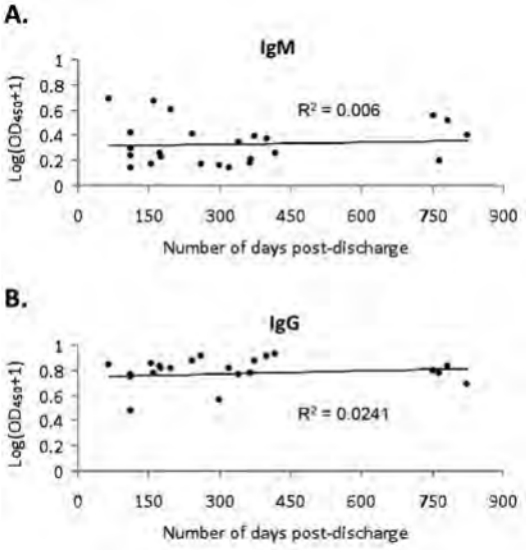
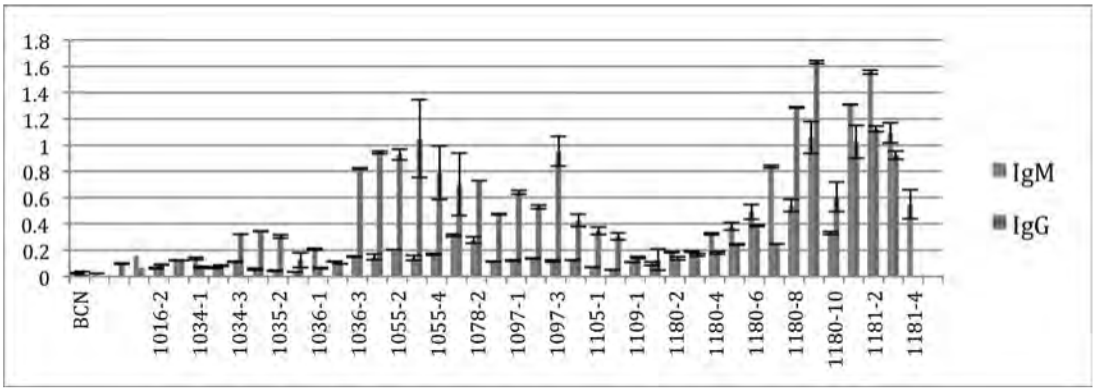


Fig. 43. Regression analysis for IgM and IgG responses against number of days post-discharge for convalescent LF patients. Background-corrected mean OD_{450} values for LASV-specific IgM (A) and IgG (B).

The kinetics of IgM- and IgG-responses in LASV acutely-infected, convalescent, and previously-exposed persons for identified epitopes on GP, NP and Z will be defined in Milestone 5 using variations of LF ELISA and ELISPOT assays. A set of appropriately cloned, mutated and expressed LASV proteins and other cross-reactive proteins (ie. Junin virus proteins) and synthetic peptides assembled under Milestone 2-4, will allow an evaluation of the reactivities to particular epitopes, or a panel of epitopes representative of divergent antigenic sites on the proteins of LASV and other arenaviruses. The analysis of immunodominance patterns of various B cell epitopes on the population level will be crucial for the identification of epitopes that are frequently targeted in a population of individuals with LASV infection.

A. IgM and IgG reactivity to LASV NP



B. IgM and IgG reactivity to LASV Z

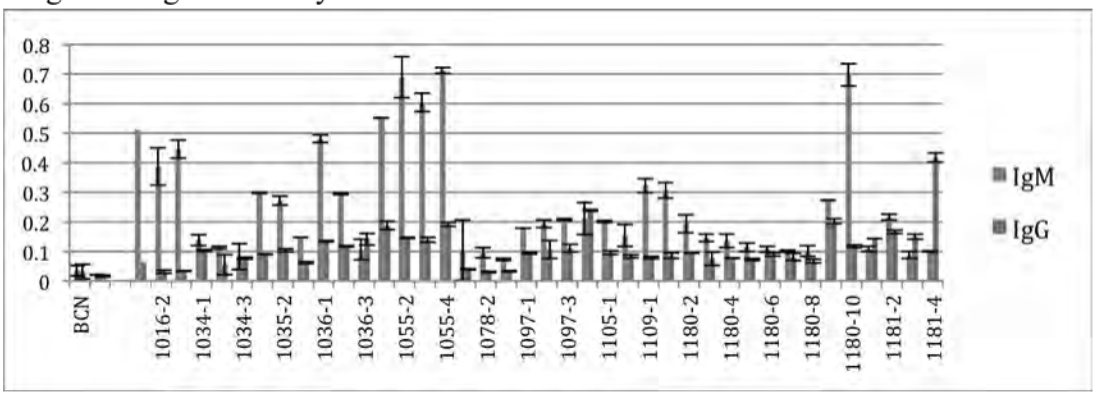


Fig. 44. Evidence for inhibition of class switching in Lassa fever infection. An identical set of LF patients and controls were analyzed in Panel A and B (note that only every other patient number is displayed).

Milestone 6: Quantify and characterize anti-LASV neutralizing and enhancing antibody responses in a well-characterized cohort of persons exposed to diverse strains of LASV at different stages and with different severities of illness (The University of Texas Medical Branch at Galveston).

Goals:

- Define cross-neutralization between LASV Old/New World arenaviruses.
- Quantify neutralizing/enhancing titers of >50 anti-Lassa MAbs.
- Quantify LASV neutralization/ enhancement in sera from 50 LF cases /Quarter
- Define mechanism of neutralization/enhancement for >50 MAbs.

OVERVIEW OF CONTRACT DELIVERABLES ACHIEVED IN MILESTONE 6.
We fulfilled modified Milestone 6 contract goals.

- We have performed extensive studies to define cross-neutralization between LASV and Old/New World arenaviruses.
- We have quantified neutralizing/enhancing titers of 120 anti-Lassa GP huMAbs, exceeding the original goal of 50.
- We quantified LASV neutralization/ enhancement in sera from approximately 50 LF cases. Since the vast majority of cases produced low or no levels of neutralizing antibodies at the time of release from the Lassa Ward, we deprioritized this goal.
- We are actively characterizing the mechanism of neutralization by huMAbs. No evidence for enhancement has been obtained.

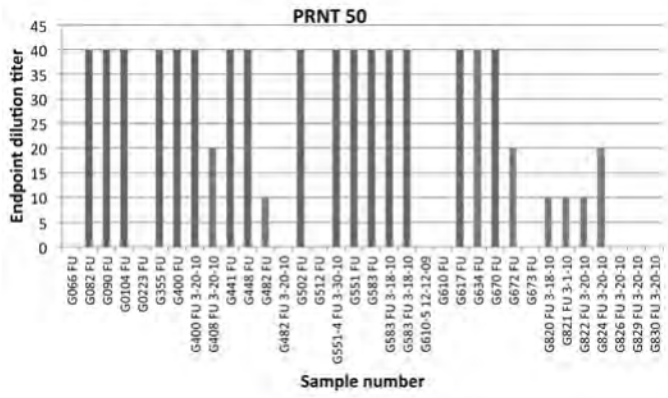


Fig. 45. Neutralizing antibody responses in Lassa fever survivors. Red bars are endpoints >1:40. Data from Joan and Tom Geisbert.

LASV neutralizing huMAbs.

We use three assays to test MAbs for neutralizing activity. The first is standard plaque reduction neutralization assay with wild type virus which was performed in a BSL-4 facility. Neutralization assays were performed by measuring plaque reduction in a constant virus:serum dilution format. Briefly, a standard amount of Lassa virus (approximately 100 PFU) was incubated with serial 2-fold dilutions of the monoclonal antibody for 60 minutes. The mixture was used to inoculate Vero cells for 60 minutes. Cells were overlaid with an agar medium and incubated for 4 to 6 days. Plaques were counted 24 hours after neutral red staining. Endpoint titers were determined by the dilution of antibody which neutralized 50% of the plaques. We used a low passage LASV Josiah virus stock, which was kindly provided by Dr. Tom Ksiazek, UTMB-Galveston, and originated from CDC Lassa-Josiah CDC number 057562. This strain was originally isolated from a human clinical specimen that was passed once in Vero cells and once in Vero E-6 cells. We passed it once in Vero E-6 cells at UTMB. The stock is a Vero p1, Vero E-6 p2. We have confirmed that it is Lassa-Josiah by positive RT-PCR for Lassa-Josiah NP. We have also tested it for mycoplasma and it is negative. The titer of this seed stock is

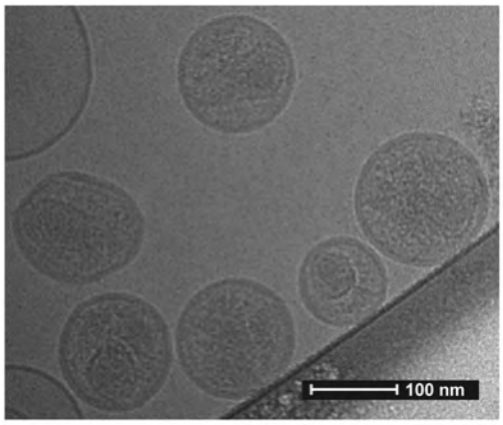


Fig. 46. LASV pseudoparticles. The characteristic "cigar-shaped" core of HIV is evident. LASV glycoproteins are present on the cell surface, albeit at lower levels than in LASV virions.

1.32 x 10^7 pfu/ml. Most LF patients that survive infection do not produce LASV neutralizing antibodies at the time of their discharge from the Lassa Ward at KGH. We used the BSL-4 neutralizing assay to determine that only about half of these LF survivors ever produce a significant tier of neutralizing antibodies and this develops only months after discharge (Fig. 45).

To circumvent working with BSL-4 pathogens, we sought ways to perform neutralization studies under BSL-2 conditions. To that end we adapted a previously described arenavirus pseudovirus assay [41]. In this method assay pseudovirion particles were produced by co-transfection of plasmids expressing arenavirus glycoproteins and a Moloney murine leukemia virus (MLV) plasmid containing luciferase reporter genes [41]. For our studies we substituted the env-deficient HIV-1 backbone plasmid, pSG3Δenv, in the co-transfection step to generate pseudoviruses containing LASV-GP as has been done for HIV-1 pseudoviruses [42] which are capable of a single round of replication. We generated LASV Josiah pseudovirions by co-transfection of pCAGGS-LASV-GPJosiah [43] and pSG3Δenv) [42] into 293T cells (Fig. 46). Transfection conditions were the same as those used to produce HIV-1Env pseudoviruses with pSG3Δenv [42]. At 72 h after transfection, supernatant fluids containing pseudovirus were harvested, clarified, filtered, and frozen in aliquots at -80 C for later use. Infectivity of LASV pseudovirus stocks were assayed in TZMbl (Hela) cells containing a firefly luciferase reporter system (Wei et al) in which reporter reporter genes are under regulatory control of an HIV-1 LTR which is activated by HIV-1tat after virus entry [42]. Although DEAE-Dextran greatly enhances HIV pseudovirus infectivity of TZM cells, it was not necessary for assay of LASV pseudovirus infectivity.

To perform neutralization assays we incubated serially diluted MAbs with pre-titered LASV pseudovirus for 1 hour. The mixture is added to 96 culture wells containing TZMbl cells at 5X10^3 cells/well. After 48-72 of culture, luciferase activit is quantified using a commercially available kit (Promega BriteGlo). Neutralization activity was assessed as a reduction in luciferase activity after a single round infection of TZM-bl cells as previously described [44].

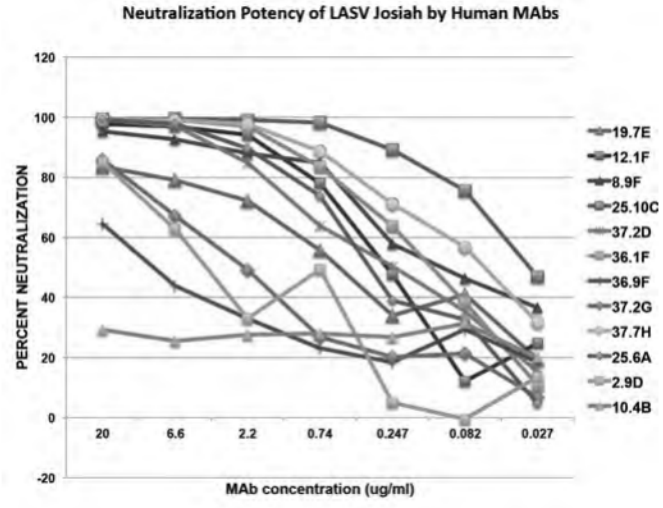


Fig 47. Neutralizing Activity of HuMAbs vs LASV PP (Josiah strain of LASV GP, lineage IV).

Testing of a group of 30 huMAbs by plaque reduction neutralization assay in BSL-4 PRNT with WT LASV Josiah identified two huMAbs, 19.7E and

10.4B, as neutralizing (Data from Joan and Tom Geisbert); however the ICD50 for these huMAbs was quite high (Fig. 47, Table 5). To facilitate screening for neutralizing huMAbs we developed an in-house neutralization assay that only required BSL-2 conditions. Rojek et al [41, 45] had developed a reporter assay for pseudovirus particles produced by co-transfecting plasmids expressing arenavirus GPs and a MLV backbone plasmid carrying reporter genes. Using the pCAGGS- LASV GP plasmid obtained from Andrew Lee at Scripps, we constructed pseudotyped LASV particles by substituting pSG3Δenv [42, 46] in the co-transfection step to generate particles containing LASV-GP and the env-deficient HIV-1 backbone as has been done for generating HIV-1 pseudoviruses [42, 46]. We had already been using pSG3 Δenv for several years for preparing single cycle HIV-1 pseudoviruses for neutralization assays. We assayed the LASVpp in TZM-bl cells, which are derived from Hela cells and contain integrated luciferase and beta-galactosidase genes under regulatory control of an HIV-1 LTR that is activated by HIV-1tat after virus entry [42, 46]. It was quite gratifying to observe that LASVpp showed very nice infectivity patterns in TZMbl cells. We then tested a panel of HuMAbs for neutralization with these pseudoviruses and observed that one of the two huMAbs, 19.7E, that had shown neutralizing activity against WT virus exhibited significant inhibition of LASVpp (Fig. 47, Table 5) as evidenced by a marked reduction in RLU (Table 5)

We have now tested almost all of our huMAbs for neutralizing activity in this assay and to date have identified 12 huMAbs that have neutralizing activity in the LASVpp reporter assay (Fig. 47). Seven of the huMAbs (25.10C, 37.7H, 37.2D, 25.6A, 36.1F, 37.2G, and 36.9F), all reacting with GP2, were derived from one subject. These huMAbs vary in potency with huMAb 25.10C showing the greatest activity, with ~78% inhibition at 0.082 µg/ml. MAbs 37.7H and 36.1F are next in potency, followed by 37.2D. MAbs 19.7E and 10.4B, also from a single donor, recognize GP1. 19.7E is not remarkably potent, and 10.4B has no activity at all in the LASVpp assay. MAb 8.9F, shows significant inhibitory activity across a range of concentrations, although it never exceeds 90% inhibition. It is noteworthy that 8.9F appears to recognize a quaternary epitope and it may neutralize by a different mechanism from the other huMAbs. In other sections of this progress report we describe experiments wherein several huMAbs were tested for passive protection of guinea pigs against challenge with lethal infection with WT LASV Josiah. Although 2 huMAbs, 19.7E and 37.2D mediated nearly complete protection and partial protection was observed with 10.4B, the huMAbs used in these experiments were clearly not our most potent. Also 19.7E protected 3 of 4 non-human primates against lethal challenge with WT LASV. The question whether our most potent huMAbs will have greater efficacy in passive protection studies in guineas or NHP remains to be answered.

Table 5. Comparison of different neutralization assays.

huMAb[1]	Speci-ficity	LASV WT PRNT	LASV-retro pp	LASV-LCMV pp[2]	LCMV-LCMV pp[2]
19.7E	GP1	+	+	+	-
10.4B	GP1/2	+	-	-	-
37.2D	GP2	+	+	+	+
36.9F	GP2	-	+	+	+
37.7H	GP2	tbd	+	+	-
37.2G	GP2	tbd	+	+	+
36.1F	GP2	tbd	+	+	-
25.6A	GP2	tbd	+	+	-
25.10C	GP2	tbd	+	+	-
2.6D	GP2	tbd	+	tbd	tbd
8.9F[3]	GP2	tbd	+	+	-

[1]37,2D, 36.9F, 37.7H, 37.2G, 36.1H, 25.6A, 25.10C, 2.9D were derived from the same subject. 19.7E and 8.9F were from two different subjects.

LCMVpp assays performed by Luis Martinez-Sobrido, Univ Rochester Med Center.

[3]8.9F neutralizes but does not bind rGP – presumably recognizes a quaternary epitope.

To address the possibility that our retrovirus based pseudotyped virus assay might not reflect valid neutralization activity, we enlisted the collaboration of Luis Martinez-Sobrido at

University of Rochester Medical Center, who has developed a different pseudovirus assay in which the backbone core is rLCMVDGP containing a GFP reporter. This plasmid can be pseudotyped with pathogenic arenaviruses to create virus particles for measuring neutralizing activity in a BLS-2 facility. Dr. Martinez tested blinded samples of a panel of neutralizing and non-non-neutralizing huMAbs. His results with his LASV/LCMV-GFP pseudovirus assay agreed 100% with ours results. He was also able to show that some, not all, subgroup GP2-A1, A2 and C huMAbs could also neutralize LCMV (Table 5, a more extensive dataset from Dr. Martinez was presented in Fig. 24).

Lassa virus strains are commonly divided into four lineages. Lineages II and III circulate in Nigeria, whereas lineage IV is prevalent in Sierra Leone and surrounding endemic regions. Lineage I, to which the first strain discovered belongs (Pinneo) does not commonly circulate in endemic areas. There is significant divergence among strains at the amino acid level for all 4 genes coded by the arenaviral genome. Of particular importance to this project, the glycoprotein complex that yields GP1, GP2, and the stable signal peptide, commonly display inter- and intra-strain variations, which could augment the challenge of developing broadly neutralizing and/or otherwise protective panels of antibodies.

We have made pseudoviruses of three other strains, LASV Pinneo (lineage I), LAS 237 (lineage II), LASV A19 (lineage III), which have been used to test the breadth of neutralizing activity of our MAbs. These studies confirmed our mapping of several epitopes. The huMAb 10.4B, which has a low neutralizing activity against wild LASV Josiah strain (lineage 4), did not display significant potential in against any LASV lineage (Fig. 48A). Four of the neutralizing huMAbs displayed high cross-neutralization against all four LASV lineages. One of these was a recently identified GP1 huMAbs 12.1F (Fig. 48C). Two of the other broadly neutralizing huMabs were 25.10C and 37.2D belong to group GP2-A1, the epitope in the fusion loop (Fig. 48C and F). 8.9F is a quaternary epitope that is neutralizes LASVpp of all lineages with high efficiency (Fig 48D), but does not react with purified GP1 or 2.

The neutralization patterns of certain of the huMAbs confirm the location of the mapped epitopes. 19.7E (GP1-A) neutralizes LASVpp of lineages 1, II and IV, but does not effectively neutralize LASVpp with the LASV lineage III glycoprotein (Fig. 48B). Key residues of the GP1-A epitope in LASV lineage IV, which is the only lineage thus far detected in Sierra Leone are SIINHKF (amino acids: 111-117) (Fig. 49). This sequence is identical in the glycoproteins of lineages I and III LASV strains that were used to prepare the pp. However, in the lineage III glycoprotein the sequence is divergent SLLHHKF accounting for the failure of 19.7E to neutralize. The failure of 36.1F (GP2-A1) to neutralize LASVpp with glycoproteins of lineages I-III can also be accounted for by variation in key residues of the epitope (Fig. 48E). In lineage IV GP the sequence is EGKDTPGGY (amino acids: 270-278), however, in the lineage I-II the sequence is EGNETPGGY (the proline following the NET indicates that this is not a glycosylation site, Fig. 49). These results also suggest that 25.10C and 37.2D the other two broadly reacting huMAbs in group GP2-A1 (Fig. 48 D and E) likely recognize an overlapping but different sequence or interact in an alternative binding configuration not involved the divergent residues.

The neutralizing antibodies 2.9D, 36.9F, 25.6A and 37.7H are derived the same subject and all fall into group GP2-A2, with the epitope mapped to the T loop. 2.9D is highly similar to 36.9F and 25.6A is highly similar to 37.7H (Tables 1 and 2). 2.9D, 36.9F, 25.6A and 37.7H neutralize lineage I LASVpp with reduced efficiency (Fig. 42H, K, I, L). The T loop diverges in slightly in part of the putative epitope. The sequence is TGRTSLPRCWV (amino acids 377-388) in lineage IV LASV GP, but is SGRTSLPKCWI in the LASV GP used in the lineage I LASVpp.

The epitope of 37.2G (GP2-C) has also been shown to be sensitive to T-loop mutations), which accounts for the somewhat lower neutralization of this antibody against the lineage I LASVpp (Fig. 48J).

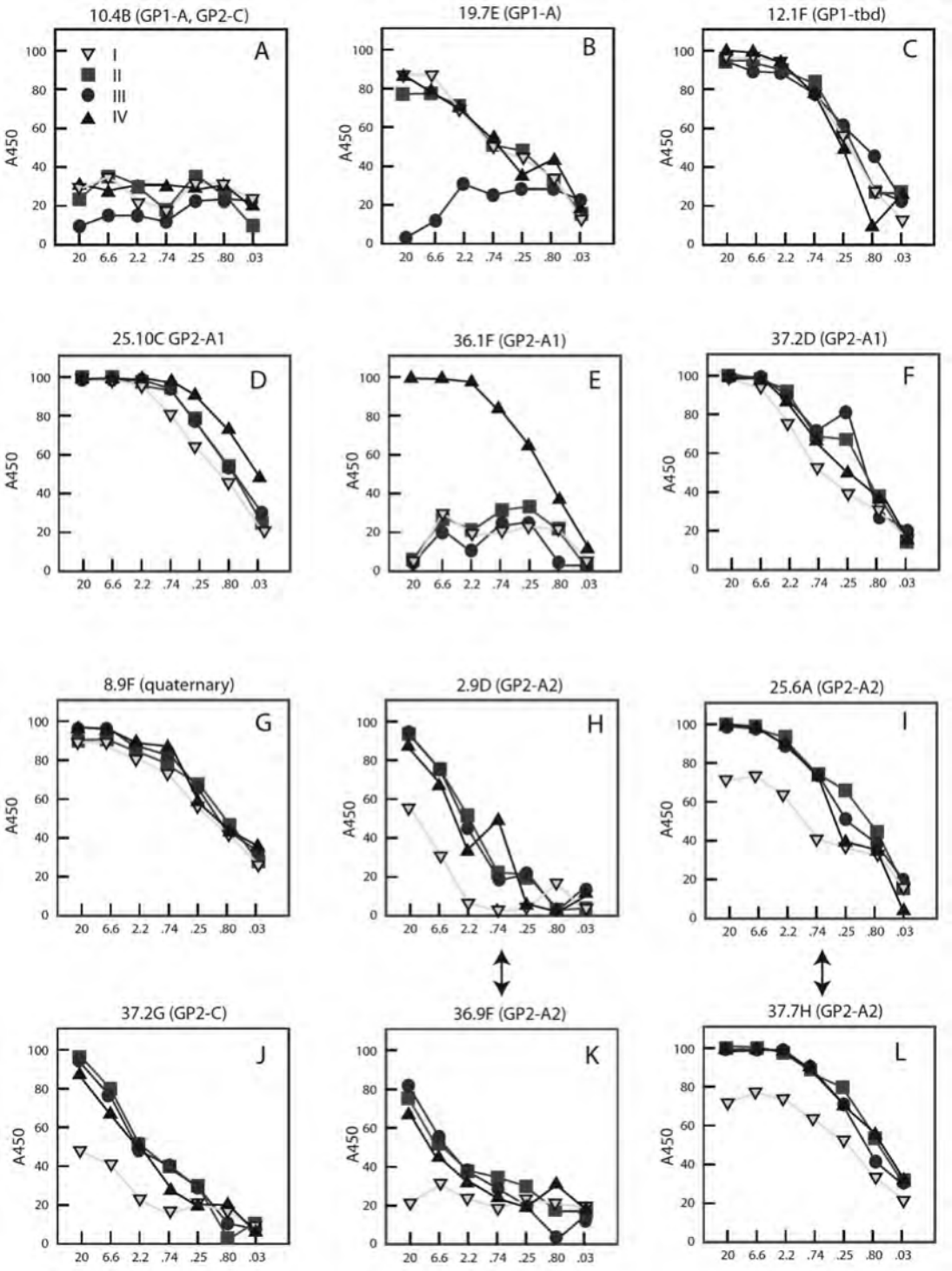


Figure 48. Neutralization by huMAbs of LASV pseudoparticles expressing glycoproteins of LASV lineages I-IV.

Fig. 49. Alignment of the amino acid sequences of the glycoproteins used to prepare the lineage I-IV LASVpp utilized in Fig. 42. Sequence variation in lineage III correlates with inability of 19.7E to block (Fig. 42B). Note that NETP in the GP2-A1 epitope does not appear to be glycosylated. Variation in lineage I-III versus IV accounts for inability of 36.1F to neutralize I-III (Fig. 42E). Reduced binding of GP2-A2 antibodies to lineage 1 LASVpp may be accounted for minor seq variation in lineage 1 (Fig. 42H, I, K, L). The binding of 37.2G has also been shown to be impacted by T loop mutations, which may account for reduced neutralization of the lineage I LASVpp.

In previous progress reports we have described huMAb 8.9F which is unusual in that appears to bind a quaternary epitope; that is, it exhibits broadly reactive neutralizing activity but does not bind to purfied LASV GP to which all other LASV huMAb produced to date bind in ELISA. Hence it is assumed it must bind to complex epitope on native envelope on virions. We recently found that this huMAb binds to native LASV GP expressed on cell surface of 293T cells transfected with our LASV GPC expression plasmid (same as used to make pseudovirus) and detection is possible by immunofluorescence (Fig. 50). Mutagenesis experiments suggest that the epitope recognized by 8.9F is located within GP1.

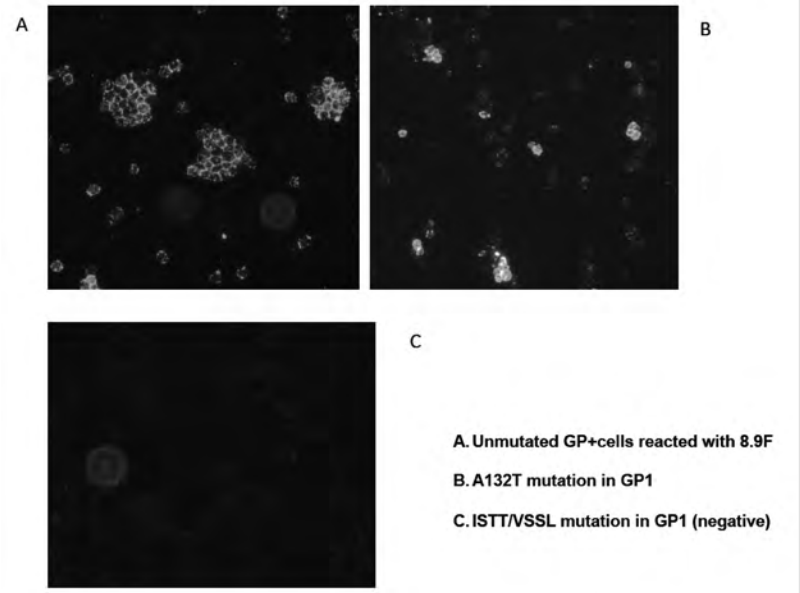


Fig. 50. 293T cells were transfected with LAS Jos GPC plasmid for ~ 18 hours. Cells were dispersed and reacted with 8.9F for 30 min. Cells were washed and reacted with FITC conjugated anti-human IgG for 30 min. After wash step, slides were covered with PBS-Glycerol (50-50) and a coveslip and examined for immunofluorescence.

C. ANTIBODY PROTECTION/ PATHOGENESIS

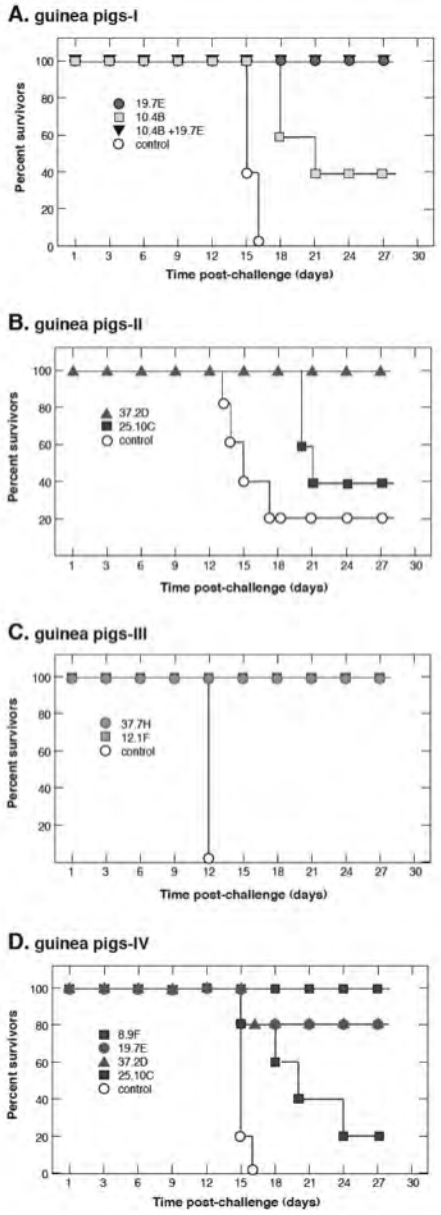


Fig. 51. Protection from lethal challenge with Lassa virus in guinea pigs by injection of single Lassa virus human monoclonal antibodies. Panel A: Survival curves for GPs treated with 30 mg/Kg and 15 mg/Kg of 19.7E and 10.4B, or 30 mg/Kg of 19.7E, 10.4B, and 37.2D individually. Panel B: Survival curves for GPs treated with 30 mg/Kg of 25.10C and 37.2D individually. Panel C Survival curves for GPs treated with 30 mg/Kg of 37.7H and 12.1F individually. Panel D: Survival curves for GPs treated with 30 mg/Kg of 8.9F, 19.7E, 37.2D and 25.10 individually.

Milestone 7: Validate protective or pathogenic role of antibodies to defined epitopes on GP, NP and Z proteins of LASV using *in vivo* challenge and protection studies in our well-established guinea pig and non-human primate models of LF (UTMB).

Goals:

- Decay/tolerance of anti-LASV MAbs in guinea pigs - Define parameters for 4-6 LASV MAbs
- Protection from death or significant disease in guinea pigs
- Define optimal MAb(s) for protection in guinea pigs
- Decay/tolerance of anti-LASV MAbs in macaques - Define parameters for 2 MAbs or MAb combinations
- Protection from death or disease in rhesus macaques
- Define optimal MAb(s) for protection in macaques

OVERVIEW OF CONTRACT DELIVERABLES ACHIEVED IN MILESTONE 7.

We exceeded each of the Milestone 7 goals.

- Decay/tolerance of anti-LASV MAbs in guinea pigs is in process and will be completed for 7 huMAbs.
- We achieved 90-100% protection from death in guinea pigs with huMabs 19.7E, 37.2D, 37.7H, 8.9F and 12.1F. Partial protection of 20-40% was achieved with huMabs 10.4B and 25.10C.
- We defined huMabs 19.7E, 37.2D, 37.7H, 8.9F and 12.1F as optimal MAb(s) for protection in guinea pigs.
- Decay/tolerance of 4 anti-LASV MAbs in macaques is in process and will be completed.
- 100% Protection from death or

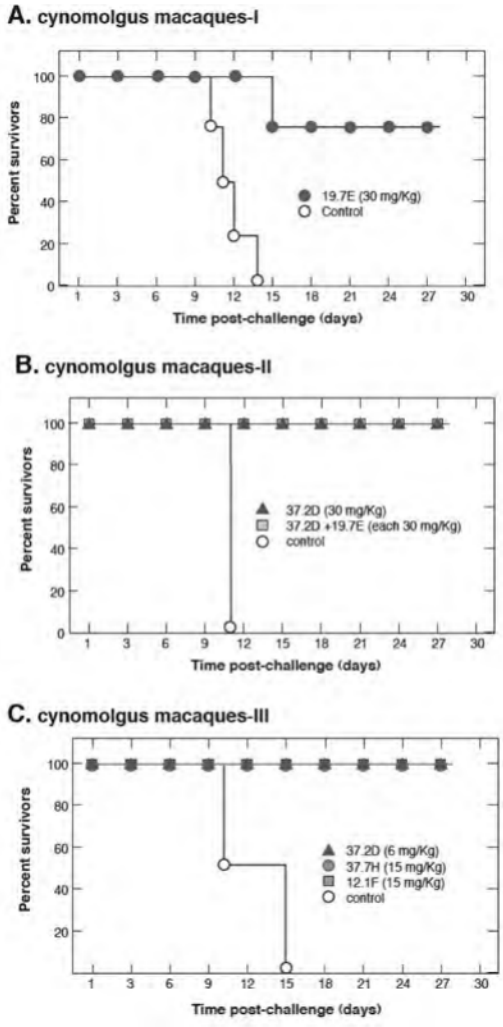


Fig. 52. Protection from lethal challenge with Lassa virus in *Cynomolgus macaques* by injection of single Lassa virus human monoclonal antibodies. Panel A: Survival curves for Cynomolgus macaques treated with 30 mg/Kg of 19.7E. Panel B: Survival curves for Cynomolgus macaques treated with 30 mg/Kg each of 37.2D and 19.7E or 30 mg/Kg of 37.2D individually. Panel C: Survival curves for GPs treated with 6 mg/Kg of 37.2D or 15 mg/Kg of 37.7H or 12.1F individually. In each study Cynomolgus macaques were challenged with a lethal dose of Lassa virus.

disease in rhesus macaques was achieved with 37.2D, 37.7H and 12.1F. 75% protection was achieved with 19.7E.

• Define optimal MAb(s) for protection in macaques. 37.2D protected 100% of macaques with a dose of 6 mg/Kg as a single antibody. Other dose finding studies will be completed as well as studies to define if delayed treatment is also protective.

Evaluation of the protective efficacy of huMAbs in animal models of LF. Human MAbs against LASV were developed by Drs. Robinson, Garry, and Branco and initially screened using a LASV pseudovirus particle assay. Promising huMAbs were then assessed *in vitro* against infectious LASV in the GNL BSL-4 laboratory. These studies identified three antibodies with substantial antiviral activity against LASV Josiah. To pilot whether these huMAbs may serve as an effective immunotherapeutic we injected outbred guinea pigs with a single dose of 30 mg/Kg and 15 mg/Kg of 19.7E and 10.4B, respectively, as a combination, on the same day as LASV challenge. We have adapted LASV Josiah to outbred GP resulting in a uniformly lethal model by the intraperitoneal (i.p.) route (Geisbert, unpublished). These outbred GP display clinical signs of the disease similar to those observed in the inbred GP strain 13, NHP, and humans. 100% of GP (4/4) in the control group injected with antibody diluent had succumbed by day 16 post-infection, whereas none of the guinea pigs (4/4) in the combined 19.7E and 10.4B treated group displayed any signs or symptoms of LF (Fig. 51A). In a follow-up study 25.10C and 37.2D were tested individually at 30 mg/Kg, and administered on the same day as LASV challenge. Two animals in the 37.2D treated group (2/5) developed transient signs of illness, but all animals recovered (51B. Three animals in the 25.10C treated group succumbed to LF between days 20 and 21 post-challenge, with the remaining 2 animals surviving without developing signs of infection. 25.10C and 37.2D were isolated from the same individual and are highly related (Fig. 54). A completely protective antibody appears to have “evolved” from a partially protective antibody by somatic mutation. Two other neutralizing antibodies 37.7H and 12.1F also provided 100% protection in the guinea pig model of LF. (Fig. 51C). The studies were repeated for several of the antibodies

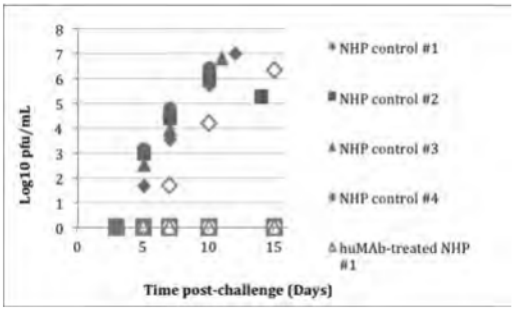


Fig. 53. Viremia levels assessed over 15 days in untreated controls or humAb 19.7E-treated macaques challenged with a lethal dose of LASV. Untreated macaques registered virus titers by 5 days post challenge. All surviving animals did not register a virus titer at any time during the study. The only animal to succumb in the huMAb-treated group first registered a virus titer on day 7 post infection, which increased steadily through day 15, corresponding to the day the animal died.

with similar results (Fig. 51D). Another antibody 8.9F also showed 100% protection of guinea pigs in this experiment (Fig. 51D).

The 40% protection level achieved with a single administration of huMAb 10.4B resulted in the highest levels of viremia in the blood of challenged animals, with 2.574 ± 0.407 Log10 pfu/mL on day 7 post infection. In contrast, 19.7E treated GP were fully protected from lethal challenge, without signs of febrile illness, despite generating titers of 1.528 ± 0.936 Log10 pfu/mL by day 7 post infection (Fig. 51A). Surprisingly, and despite transient fever and lethargy in some animals treated with 37.2D, all GP in this group did not register detectable infectious virus on day 7 of the study (Fig 51B). THE correlation between presence of detectable viremia held in the subsequent guinea pigs experiments (51C,D). All of the animals that succumbed showed detectable viremia. All the animals that survived showed no viremia, or in two animals (37.2D) transient low level viremia.

A study of *Cynomolgus macaques* challenged with a lethal dose of LASV Josiah, followed by administration of 30 mg/Kg of 19.7E alone, at days 0 and 5 post infection, protected 75% of animals (3/4) (Fig. 52A). One animal succumbed on day 15 of the study. Control animals challenged with the same LASV strain succumbed on days 10, 11, 12, and 14. 100% Protection from death or disease in rhesus macaques was achieved with either 37.2D alone or in combination with 19.7E (Fig. 52B). 37.2D was also 100% protective in the Cynomolgus macaque model of LF at a dose of 6 mg/Kg, whereas 37.7H and 12.1F were 100% protective at 15 mg/Kg (Fig. 52C).

In NHP, 3 out of 4 animals that survived challenge with a lethal dose of LASV after a single administration of 19.7E did not register a blood virus titer throughout the course of the study (Fig. 53). The single macaque that succumbed to LASV, despite receiving an identical dose of huMAb 19.7E and the same lethal dose of virus, started to register a viral titer by day 7, which increased through day 15, at which time the animal succumbed to the illness (Fig. 52A). These results indicate that huMAb

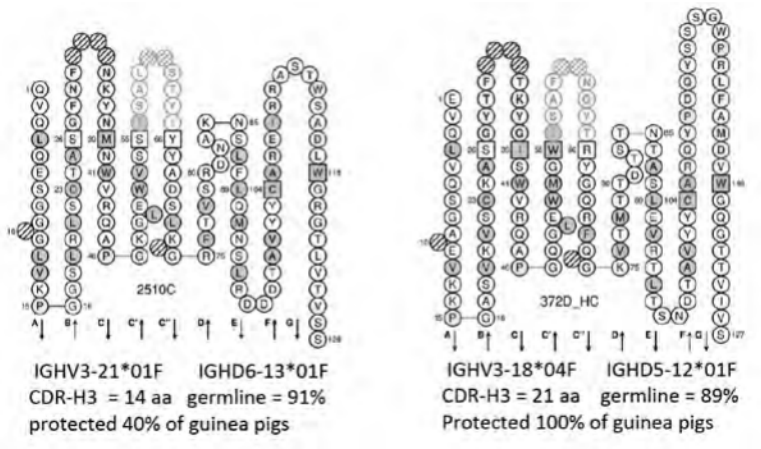


Fig. 54. Opportunities to define correlates of immunoprotection to Lassa fever with huMAbs 37.2D or 25.10C. Two closely related huMabs from the same patient give different levels of protection in guinea pig model.

19.7E alone confers a high degree of protection against infection with a lethal dose of LASV, when administered at the time of infection, both in GPs and NHPs. No detectable infectious virus was detected in circulation of Cynomolgus macaques treated with 37.2D (Fig. 52B, C) even at the low dose of 6mg/Kg. Likewise, 37.7H or 12.1F treated animals (Fig. 52C) did not have infectious LASV in their blood.

Also of interest to this contract is that Luis Branco, PhD .Zalgen Labs LLC, is the inventor of a newly patented mammalian cell-based biomanufacturing system, CHOL*Celect* (U.S. Patent US8076102), designed to generate highly regulatory compliant NS0 stable cell lines in less than half the time of classical platforms (Fig. 55). This system utilizes a novel metabolic selection gene that rescues a cholesterol biosynthetic pathway auxotrophy in parental NS0 cells. This system is employed in the biomanufacturing of Zalgen Labs' recombinant protein products. CHOL*Celect* is ideal for generation of immunotherapeutics for resource limited settings such as West Africa, given that ownership of the manufacturing system will eliminate payment of licensing fees and milestone driven royalties to a third party entity.

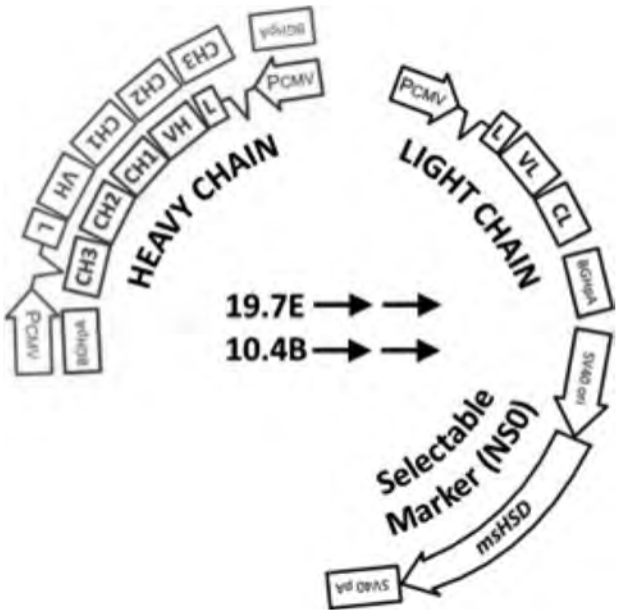


Fig. 55. Bicistronic mammalian expression vector for large scale production of therapeutic antibodies in NS0 (non-secreting null) cells. Heavy and light chain gene expression cassettes can be cloned in tandem or opposing orientations. LASV huMAbs 10.4B and 19.7E expressed at higher levels from tandem orientation constructs, which were subsequently used to generate stable production grade NS0 cells lines.

This system has several advantages over the NS0-GS system:
1. Stable cell lines can be generated from the onset in chemically defined serum-free medium (CD-SFM); 2. Medium is supplemented with highly soluble glutamine, but not cholesterol, which is commonly supplied in carbohydrate-coupled form to retain solubility; 3. Maintenance of adequate levels of unstable cholesterol complexes in the medium to support optimal cell growth during fermentation is not a concern; 4. Cell lines can be generated in approximately half the time of conventional methods, thus permitting a significant reduction in development timelines for leading candidate molecules; 5. Failure rate of stable cell line adaptation from a serum-based medium to CD-SFM is reduced to zero; 6. Potential for introduction of adventitious agents through non-defined components, such as bovine serum, is significantly reduced.

C. MECHANISMS OF ANTIBODY PROTECTION/ PATHOGENESIS

Milestone 8: Determine LASV sequence diversity and impact on recognition of B cell epitopes by LF patients (Harvard/Broad Institute).

Goals:

- Ultra high-throughput next generation sequencing of 50 to 75 complete LASV genomes per Quarter.
- Analyze geographical and temporal genetic variation, genomic substitutions, rearrangements, and recombination and the impact of LASV sequence diversity on epitope recognition and LF severity/outcome.

OVERVIEW OF CONTRACT DELIVERABLES ACHIEVED IN MILESTONE 8.
We exceeded each of the Milestone 8 contract goals.

- Using ultra high-throughput next generation sequencing we have sequenced over 300 full-length Lassa virus (LASV) genomes, which constitutes the largest genomic dataset of any BL-4 agent ever produced. In this current Quarter, we also sequenced nearly 100 Ebola virus genomes at ~2000X coverage over a 10 day period.
- As described below we have also used this data to analyze geographical and temporal genetic variation. These dataallowed is to determine the evolutionary history of LASV and found that it originated in modern-day Nigeria more than 1,000 years ago. Wehave also genomic substitutions, rearrangements, and recombination. We are completing studies that demonstrate the impact of LASV sequence diversity on epitope recognition and LF severity/outcome. Non-synonymous intrahost mutations in LASV accumulate in B cell epitopes in the glycoprotein.

Success in this Milestone 8 required development of several new protocols and technologies that enabled us to massively increase our ability to sequence viral genomes. We have submitted several manuscripts based on these datasets where we present our significant technological advances and important scientific insights into Lassa and Ebola virus biology and evolution.

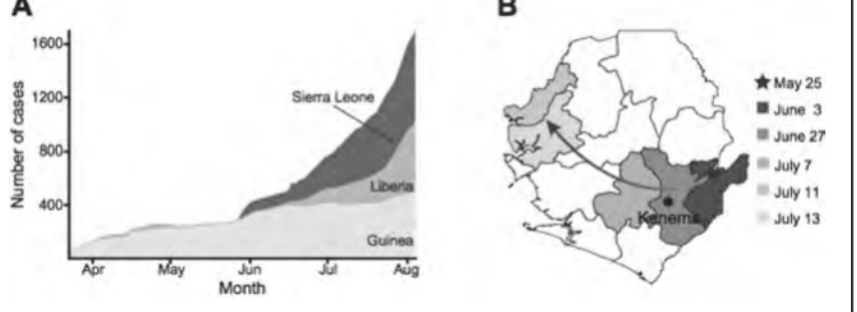


Figure 56: (A) 2014 outbreak growth fits an exponential growth curve (R2=0.94) with a doubling time of 36 days (confirmed, probable and suspected cases are considered). (B) Generalized temporal spread of EVD in Sierra Leone by district. The gradient denotes the timing of spread and the arrows depicts the likely direction.

A devastating outbreak of Ebola virus disease (EVD) in West Africa is currently wrecking havoc in Guinea, Liberia, Sierra Leone and Nigeria with more than 1,700 cases (as of August 8th, 2014) in what is by far the largest outbreak of EVD ever recorded (**Fig. 56A**). Prior to the outbreak in Sierra Leone, we helped the Kenema Government Hospital (KGH) setup and thoroughly validate PCR-based diagnostics based on three different primer sets. This enabled the Sierra Leonean scientists at KGH to detect the first EVD case in the country on the 25th of May 2014. The same setup was also introduced in Nigeria, where our partner Christian Happi at Redeemers University, together with Sunday Omilabu in Lagos, similarly detected the first case of EVD on the 25th of July 2014 in that country. Despite early detection, however, the outbreak has quickly spread across Sierra

Leone (**Fig. 56B**) and the rest of West Africa, and the number of cases is continuing to increase exponentially (**Fig. 56A**; doubling time ~ 1 month).

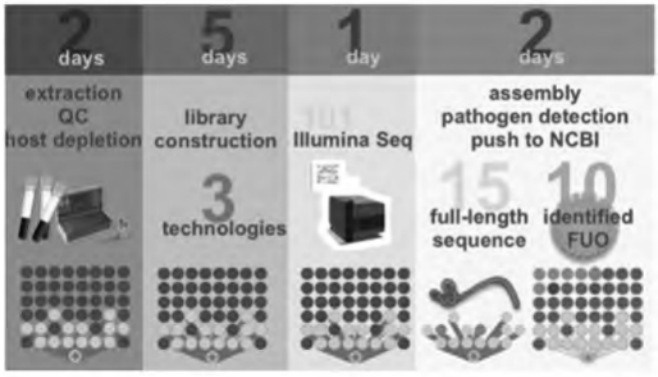


Figure 57: Overview over our 10-day processing time from sequencing to complete viral genome assembly.

In order to gain critical insights into this rapidly developing outbreak, we expanded our collaboration with the Sierra Leone Ministry of Health and Sanitation. Quickly after the EVD outbreak had started in Sierra Leone, we received a batch of 12 patient samples and sequenced (using three different technologies) 15 complete Ebola virus (EBOV) genomes in a turn-around time of 10 days from sample receipt to genome assembly (**Fig. 57**). We later received another batch of 66 patient samples, which we also sequenced and analyzed within ten days, providing us with the largest genomic dataset of EBOV sequences ever produced. This dataset of 99 full-length genomes constitutes ~ 94% of all EVD cases diagnosed in Sierra Leone over the first month of the outbreak in this country (**Fig. 58**). We published all sequences to NCBI as soon as they were assembled - and prior to publication - in order to give the scientific and medical communities immediate access to this valuable resource.

A

	# Total	# Patients	% Coverage	x Coverage
Batch 1	15	12	99.77%	555 [16-23,042]
Batch 2	84	66	99.91%	2,186 [6-18,623]
Combined	99	78	99.88%	2,048 [6-23,042]
Replication	140	78	99.39%	2,109 [8-17,537]

B

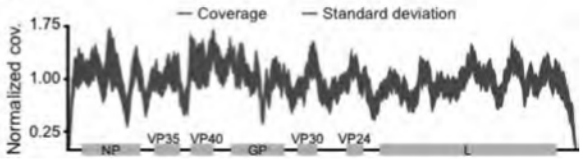


Figure 58: (A) EBOV samples from 78 patients from Sierra Leone were sequenced to a high depth of coverage. Samples were sequenced in two separate batches and some patients were sampled at multiple time points, totaling 99 viral genomes. The median depth of coverage including range, as well as the average percent coverage are shown. (B) Plot of the combined normalized (to the sample average) coverages across the EBOV genomes sequenced in this study.

2014 West African Ebola Outbreak Response
The 2014 West African Ebola strain diverged from Central Africa strains a decade ago

Based on our rich dataset of EBOV sequences, we first established the phylogeny and long-term evolutionary history of the 2014 West African outbreak strain. Using serial sampling and coalescent Bayesian molecular dating, we estimate that the current strain of EBOV split from Central African EBOV strains around a decade ago (**Fig. 59A**). We also estimated the common ancestor of all currently circulating lineages to be ~ March 2014 (**Fig. 59B**), which is fully in line with the epidemiological evidence, and suggests that the outbreak was detected relatively quickly (the date of the first confirmed case is around the time of our estimations).

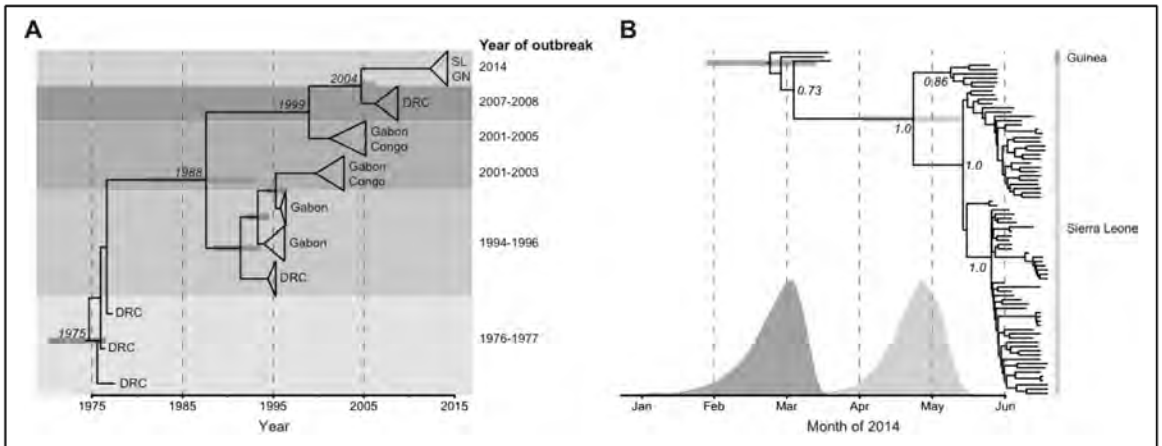


Figure 59: (A) BEAST dating of the separation of the 2014 West African strain from Central African lineages (tMRCA: Sep 2004, 95% HPD: Oct 2002 - May 2006). (B) BEAST dating of the tMRCA of the 2014 West African outbreak (tMRCA: Feb 23, 95% HPD: Jan 27 - Mar 14) and the tMRCA of the Sierra Leone lineages (tMRCA: Apr 23, 95% HPD: Apr 2 - May 13); probability distributions for both divergence events in 2014 are overlayed below. The posterior support for major nodes is shown.

We also found that all the sampled sequences followed a very predictable tree-like pattern with Guinean samples being ancestral to later Sierra Leonean sequences and later lineages building on early Sierra Leone diversity (**Fig. 59B**). This, combined with the fact that all strains

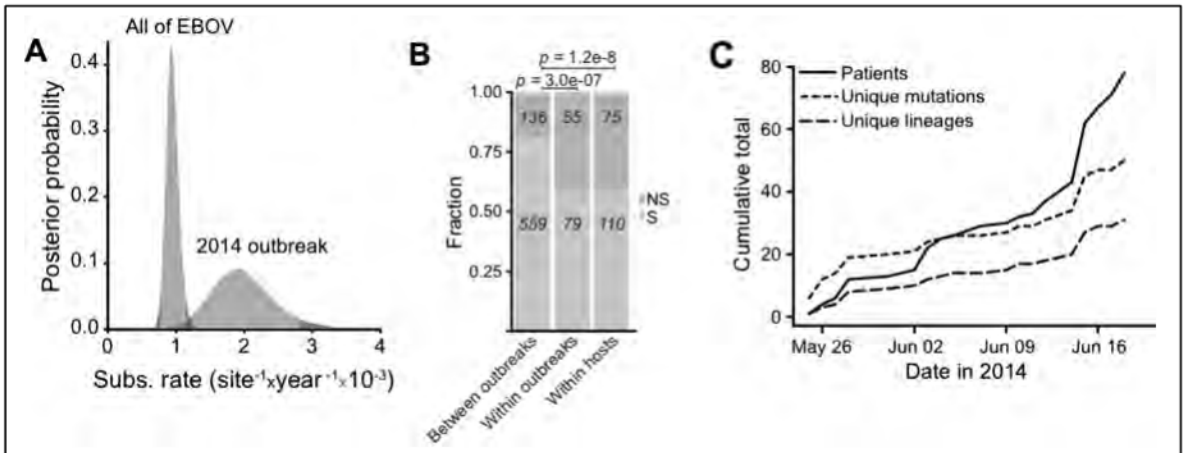


Figure 60: (A) Difference in substitution rate estimates within the 2014 outbreak and between all EVD outbreaks. (B) Different proportion of nonsynonymous changes at three timescales - between outbreaks, within outbreaks, and within hosts (S=synonymous; NS=non-synonymous). (C) Acquisition of genetic variation over three weeks' time. As the number of patients increases (solid line), we observe a total of 50 mutational events (short dashes) and the emergence of 29 new viral lineages derived from the original 2 that entered Sierra Leone (long dashes).

were very similar, strongly suggest that the current outbreak is a result of sustained human-to-human transmission, rather than multiple introduction from its zoonotic (presumably bat or monkey) reservoir. The latter would be predicted to result in no clear ladder-like structure of the

genetic data, combined with relatively high viral diversity - both of which we do not see in our dataset.

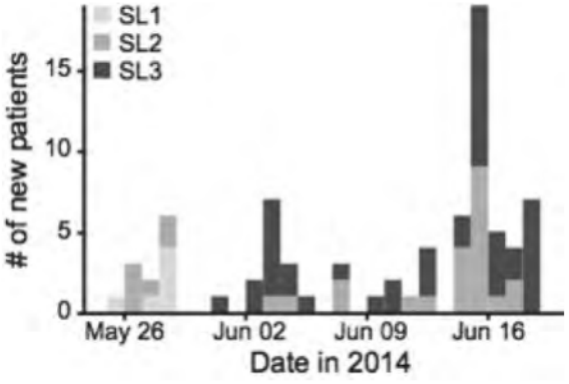


Figure 61: Number of EVD-confirmed patients per day in our study, colored by genetic cluster (SL1-3).

EBOV evolves at an increased rate during the 2014 outbreak, making rapid containment imperative

Given our deep sampling of most of the Sierra Leonean EVD cases during the early stages of the outbreak in Sierra Leone (~ 94% of all EBOV patient samples were sequenced), we were able to estimate the 'mutational spectrum' of the virus and get a better understanding of how

it evolves as it transmits from human-to-human. We found that the observed substitution rate of EBOV strains during the 2014 outbreak was significantly higher than that observed between outbreaks (**Fig. 60A**). This suggest that the virus is capable of evolving rapidly during an outbreak and we also observed that many of these changes were non-synonymous (amino acid replacement) mutations, which could presumably be of functional consequence (**Fig. 60B**). Combined with an accelerated increase of unique mutations and unique viral lineages as the outbreak progresses (**Fig. 60C**), the West African EVD outbreak may provide EBOV with a truly unique and dangerous opportunity to evolve and adapt to the human host. This is of serious concern, as the virus continues to spread rapidly in large urban areas, potentially giving the virus the ability for more efficient human-to-human transmission. This is especially disconcerting since EVD was recently detected in Lagos, Nigeria - a city of more than 20 million people and by far the largest urban center in Africa with a very busy international airport. It is important to note however, that we have no evidence to suggest that the virus is indeed adapting - we only observe that it is changing.

While we do not yet know the functional consequences (if any) of the various mutations that we observed as EBOV spread through Sierra Leone, we found that certain viral lineages appeared early in the outbreak, only to be replaced by other lineages later in the outbreak (SL1-SL3 in **Fig. 61**). For example, we observed that the lineages SL1 and SL2 were present early in the outbreak, whereas a third lineage (SL3) appeared after a week and swept through the viral population becoming the predominant - and later only - lineage observed in our patient samples (**Fig. 61**). Further support for viral sequencing and experimental validation is needed in order to better understand the viral dynamic and functional consequences of the spread of EBOV as the virus transmit from human-to-human.

Figure 62: Lassa fever endemic countries with the estimates of the LASV L segment time to the most recent common ancestor (tMRCA) for each region (median values; ya = years ago). Gray arrows depict the most likely spread of LASV across West Africa.

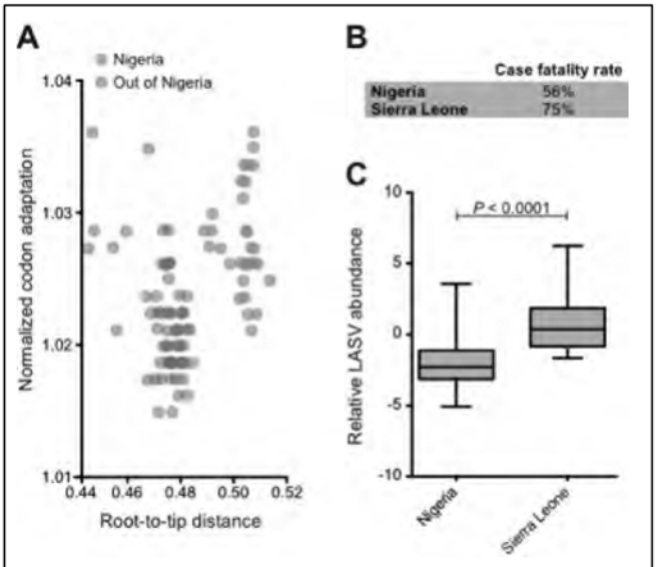


Figure 63: (A) Normalized CAI (to human) of LASV sequences plotted against their distance (aa substitutions/site) to the root of the maximum-likelihood phylogeny. (B) Case-fatality rates calculated for patients from Sierra Leone (n = 36) and Nigeria (n = 36). The rates were calculated only from patients with known outcomes. (C) Relative abundance of LASV genome copies in sequencing libraries (log ratio of LASV copies/µl to 18S rRNA copies/µl; Mann-Whitney test).

Elucidation of the evolutionary dynamic of Lassa virus and production of the largest BL-4 agent dataset

As mentioned previously, we have now sequenced more than 300 LASV genomes, which constitutes the largest genomic dataset of any BL-4 agent ever produced. We have submitted several manuscripts based on this dataset, where we present our significant technological advances and important scientific insights into Lassa virus biology and evolution.

Based on this dataset we first elucidated the evolutionary history of LASV and found that it originated in modern-day Nigeria more than 1,000 years ago. Our data also suggests that the virus spread out of Nigeria about 400 years ago and only recently entered Sierra Leone ~150 years ago (**Fig. 62**).

The LASV spread 'out-of-Nigeria' correlates with an increase in viral adaptation

We were surprised when we found that LASV had ancient origins in Nigeria, but only relatively recently appeared to have spread to other West African countries. Interestingly, we found that this spread of LASV across West Africa correlates with a significant increase in the codon adaptation of the non-Nigerian lineages (**Fig. 63A**). We performed this analysis by calculating the codon adaptation index (CAI) of LASV to its human (and rodent) hosts. CAI provides a measure of synonymous codon usage optimization for viral replication using the host translational machinery. It effectively quantifies how well the choice of synonymous codons in the viral genome matches that of the host in which it replicates, with better 'adapted' viruses presumably being able to replicate better. We found that Sierra Leonean strains had significantly higher CAIs than their Nigerian counterparts (**Fig. 63A**). Increased codon optimization of non-Nigerian strains might lead to an increase in viral protein output and therefore higher viral titers. If true, since increased viremia of LASV in LF patients is highly correlated with greater fatality rate, we would also expect CFRs to be higher amongst patients in Sierra Leone than in Nigeria. We standardized the inclusion criteria at both our field sites and examined evidence to assess this hypothesis. We used qPCR to quantify LASV genome abundance and found significantly higher numbers of LASV genomes in human samples from Sierra Leone than Nigeria (**Fig. 63C**). Similarly, using very strict criteria for inclusion in CFR calculations, we also found a markedly higher CFR in Sierra Leonean patients compared to their Nigerian counterparts (75% vs. 56%; *P*-value = 0.04, Chi-squared test; **Fig. 63B**). While these findings are consistent with the hypothesis of an association between increased CAI, increased viral loads, and increased human CFR as LASV spread out of Nigeria, we have not yet established causality. Parameter differences beyond our control -

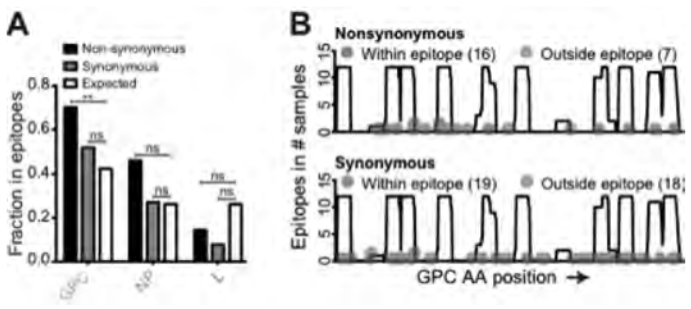


Figure 64: (A) Fraction of iSNVs falling within predicted B cell epitopes for each LASV protein (** = P < 0.01, Binomial test; ns = not significant). (B) Overlap between GPC epitopes and iSNVs. Epitopes were predicted separately in each sample (y-axis) and overlaid with iSNVs from that sample.

including variations in socio-economic, clinical and human genetic factors - prevent us from determining whether increased CAI does indeed lead to an increase in viral titers and CFRs. Further studies, including infection experiments in animal models, are required to fully address these questions.

Non-synonymous intrahost mutations in LASV accumulate in B cell epitopes in the glycoprotein

The long-term evolution of LASV ultimately involves mutation and selection within individual hosts. Our deep sequencing approach (median > 100x coverage/bp) allowed us to examine LASV intrahost single nucleotide variants (iSNVs) within individual human and rodent hosts. When we examined the various LASV genes for signatures of positive selection within individual hosts, we found an excess of nonsynonymous iSNVs in the LASV glycoprotein (GPC), the only protein partially exposed on the outside of the LASV particle. This gene has a significantly higher within-host dN/dS than NP, the neighboring gene on the S segment. We believe that this increase in dN/dS specifically targeted at GPC is driven either by a GPC-specific relaxation of within-host purifying selection, or within-host diversifying selection.

We hypothesized that immune pressure on GPC could be a driver of within-host diversifying selection for iSNVs that avoid immune detection by disrupting epitopes. This phenomenon has been reported for other viruses; for example, at the population-wide level in pandemic influenza A virus infections and at the intrahost level in chronic HIV carriers. In addition, LASV-specific antibodies appear relatively quickly, with experimentally infected non-human primates developing detectable IgM titers after nine days and IgG titers after twelve. To evaluate whether iSNVs disrupt epitopes, we used a machine learning method to predict linear B cell epitopes in each LASV protein. We found that nonsynonymous iSNVs in GPC occur in predicted epitopes significantly more than expected by chance (binomial test, $P < 0.01$; **Fig. 64A, B**), whereas synonymous iSNVs (which do not change epitope structure) are randomly distributed across the protein. This supports a role for B cell-mediated immunity in selectively shaping the intrahost viral population. We did not find a significant enrichment in epitope-iSNV overlap in any other LASV protein (**Fig. 64A**). In a very preliminary study we created constructs that expressed different subsets of LASV with different iSNV mutations. The constructs contained either subsets of the mutations. The construct contsain 6 mutations did not express in mammalian cells. The other construct Mut 2 was expressed at high levels but bound lees amounts of several huMAbs ((Fig. 65). Futher experiments will express individual mutations.

Viral Sequencing Technology Development

Development of broadly applicable viral sequencing methods

Deep massively-parallel sequencing of viral genomes from clinical samples have helped us and others to provided key insights into emergence, evolution, intra-host diversity and transmission of viral pathogens. However, the majority of clinical samples contain very little viral RNA, are heavily contaminated with human RNA, and in some instances the nucleic acids are severely degraded. While poor sample quality affects viral sequencing in general, it is

exacerbated for EBOV and LASV samples. Sample quality is compromised by delays and

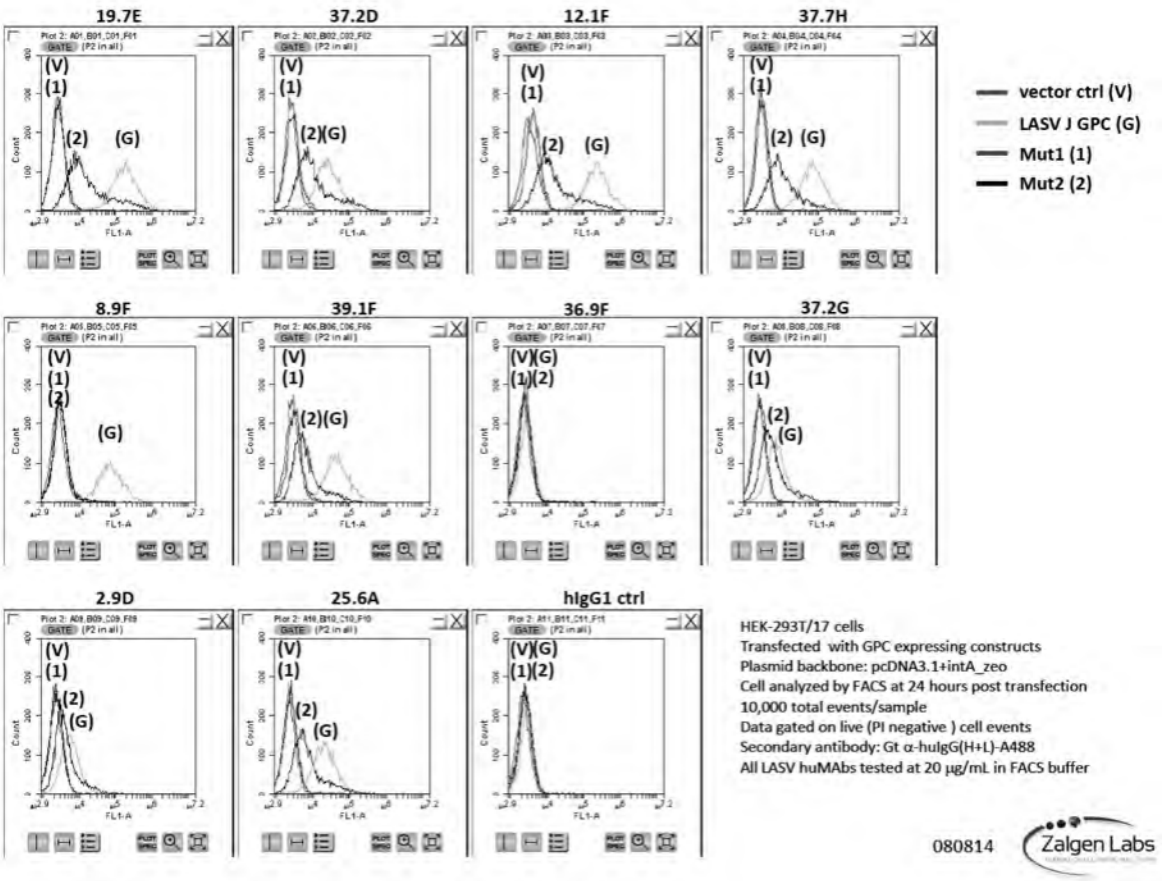


Fig. 65. Binding of huMAbs to LASB GP with various iSNV changes. Mut 1 was not expressed. Mut 2 was expressed, but bound reduced amounts of various huMAbs. This was a feasibilty experiment and will be followed with studies expressing LASV GP with single iSNV variations.

interruptions of the cold chain between sample collection in remote rural areas in hot climates, and limitations posed by issues with handling, containment and biological inactivation at the highest biosafety level (BL-4), international shipment and delivery at overseas research facilities.

To solve these issues, we developed a robust RNA-seq method for generating complete *de novo* assemblies of LASV and EBOV genomes in clinical and biological samples. Our method uses targeted RNaseH-based degradation to remove contaminating poly(rA) carrier and rRNA. This depletion step improves both the base quality and quantity of informative Illumina reads in unbiased total RNA-seq libraries. Moreover, we developed a hybrid-selection protocol to enrich the viral content of sequencing libraries even further, rescuing samples that previously did not yield complete genome assemblies. Our protocols lower sequencing costs and are broadly applicable to other viral genomics studies.

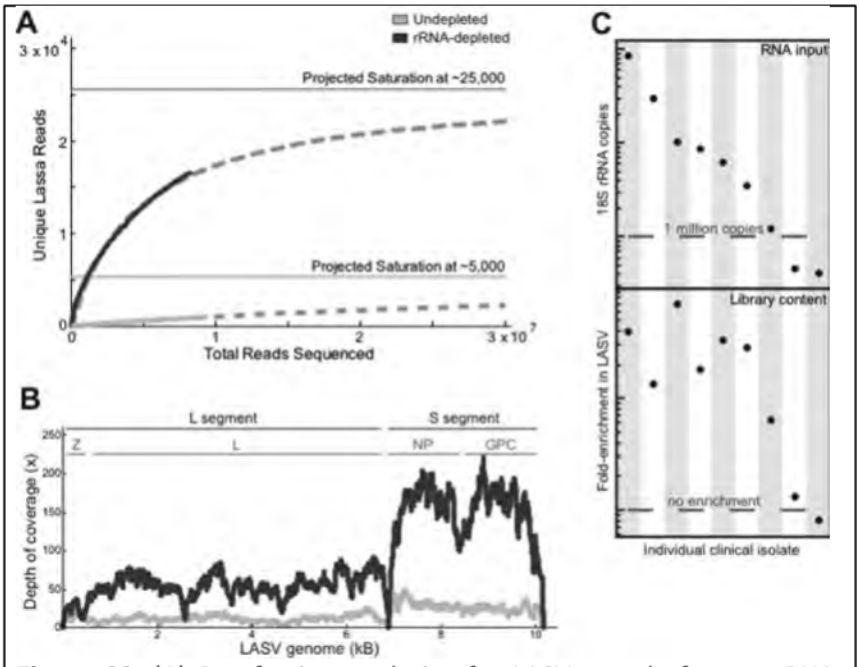


Figure 66: (A) Rarefaction analysis of a LASV sample from a rRNA-depleted (grey) or control (undepleted, blue) preparation. Data best fit (dashed line) to the Michelis-Menten formula in which projected saturation value equals Vmax (see Methods). (B) LASV genomic coverage from a LASV sample from a rRNA-depleted (grey) or control (blue) preparation. L, S segment, Z, L, NP, GPC: boundaries of each LASV genomic segment with specified genes encoded on each segment. (C) Starting overall content (RNA input) and enrichment of unique LASV (Library content) upon rRNA depletion from nine different clinical isolates.

To remove rRNA, we tested selective RNase-H-based depletion using oligodeoxyribonucleotides tiled along human cytoplasmic and mitochondrial human rRNA sequences. We achieved almost complete removal of rRNA (from ~80% of the reads down to less than 0.5%) and a concomitant eight-fold enrichment of LASV content in a human plasma sample. As shown by rarefaction analysis (**Fig. 66A**), rRNA depletion increased the unique LASV content in the sequence data to an estimated saturation at ~25,000 non-duplicated LASV reads compared to at most 5,000 without depletion. The depletion improved overall sequencing depth along the LASV genome (**Fig. 66B**) revealed finer details such as the pronounced differences in coverage between the L and S segments which are known to be present at different copy numbers in infected cells and the dip in coverage at the stem-loop secondary structure between the NP and GPC gene.

Most LASV isolates collected from human serum or plasma contain very little total RNA. To preserve as much precious RNA as possible for library construction, we used a real-time qRT-PCR assay for 18S rRNA as surrogate for quantification of total RNA. To determine the minimum amount of input RNA required for efficient LASV enrichment, we performed rRNA depletion on nine samples spanning a wide range (~200-fold difference) of input RNA. Our protocol enriched unique LASV content at least five-fold in all samples with at least one million copies of 18S rRNA (**Fig. 66C**). Thus, the rRNA selective depletion method can be applied to extremely low-input RNA samples containing as little as attomoles of rRNA. In comparison to previous RNase-H publications, our method was successful with ~1,000-fold less material.

Since our approach worked well with a wide range of clinical LASV samples we next tested it on EBOV samples from the 2014 outbreak in Sierra Leone (as described above). In the interest of speed, we used Nextera transposition instead of adapter ligation to prepare Illumina libraries from second strand cDNA. We sequenced four individual clinical isolates with and without rRNA depletion and generated approximately one million Illumina reads per library. We were able to lower the rRNA contamination in all four samples from >80% to <0.5% (**Fig. 67**). The

concomitant increase of EBOV content was approximately 13 to 24-fold, reaching ~35% of unique reads in one of the rRNA depleted libraries (**Fig. 67**).

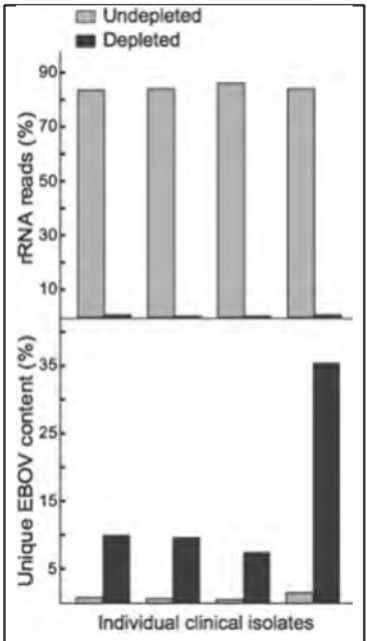


Figure 67: (A) Percentage rRNA (left) and unique EBOV content (right) with (grey) and without (blue) rRNA depletion in four individual clinical serum isolate.

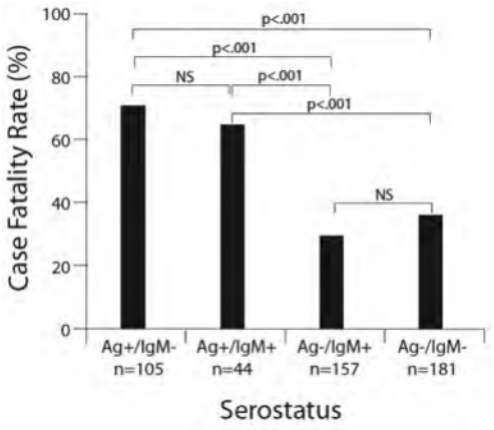


Fig. 68. CFRs in suspected LF cases presenting to the KGH Lassa Ward by serostatus, 2008-12. The presence of LASV Ag and anti-LASV IgM in serum of patients with known outcomes was assessed by recombinant Ag- and IgM capture ELISA, respectively. Statistical significance was determined using a logistic regression model predicting CFR. NS = not significant

Milestone 9: Evaluate all B cell epitopes discovered (under Requirement a) for functionality by comparing these acquired responses with LF clinical disease outcomes to identify the mechanisms of protection or pathogenesis in humans.

Goals:

- Enroll 200-300 cases of confirmed LF per year; 50-75 per Quarter.
- Correlate IgM and IgG titers, neutralizing/enhancing Ab titers, ELISPOT results to clinical outcomes and reactivity to specific B cell epitopes to clinical outcomes.

OVERVIEW OF CONTRACT DELIVERABLES ACHIEVED IN MILESTONE 9.
We will fulfill modified Milestone 9 contract goals.

- We enrolled 200-300 cases of confirmed LF per year; 50-75 per Quarter at our clinical sites at Kenema Govdernment Hospital in Sierra Leone and at Irrua Specialist Teaching Hospital (ISTH) in Nigeria.
- As described in Shaffer et al. (2014) we have correlated IgM and IgG titers to clinical outcomes in all consenting human subjects presenting to Kenema Government Hospital during the project period. We are analyzing a similar dataset collected at ISTH. AS discussed, patients with acute LF did not make significant titers of neutralizing or enhancing antibodies hence this goal was dropped. Most B cell reactivities are to NP, rather than GP epitopes and therefore the ELISPOT studies were deprioritized. Epitope-specific ELISA have been produced and we are in the process of analyzing reactivity to specific B cell epitopes and comparing these to to clinical outcomes.

The goals of this Milestone were to correlate clinical data on LF in conjunction with data on humoral immune responses, cytokine profiles and outcomes of LASV infection. A positive result on the LASV Ag-capture ELISA or lateral flow immunoassay utilized at KGH indicates that the subject is viremic, and when combined with clinical observations, can be used to confirm a diagnosis of acute LF. Among subjects with suspected LF evaluated at the KGH Lassa Ward from 2008-2012, 10.9% (191/1748) were antigenemic. These patients were also evaluated for the presence of anti-LASV IgM: 8.1% (141/1748) were Ag positive and IgM negative (Ag+/IgM-), while 2.9% (50/1748) were Ag positive and IgM positive (Ag+/IgM+). Previous studies indicated that LASV infection can dysregulate antibody class switching (conversion from a predominantly IgM response to a predominantly IgG response) and that anti-LASV IgM often persists for months to years after LASV exposure.[47] Thus, many suspected LF cases presenting with anti-LASV IgM in the absence of LASV antigenemia appear to have febrile illnesses other than LF. A total of 23.3% (408/1748) of non-antigenemic patients with suspected LF had prior exposure to LASV, as indicated by the presence of IgM antibodies. Suspected LF patients with neither LASV antigenemia nor IgM (Ag-/IgM-) were

considered to have an undiagnosed acute febrile illness (UAFI, also known as a fever of unknown origin), and accounted for 65.7% (1149/1748) of suspected LF cases evaluated.

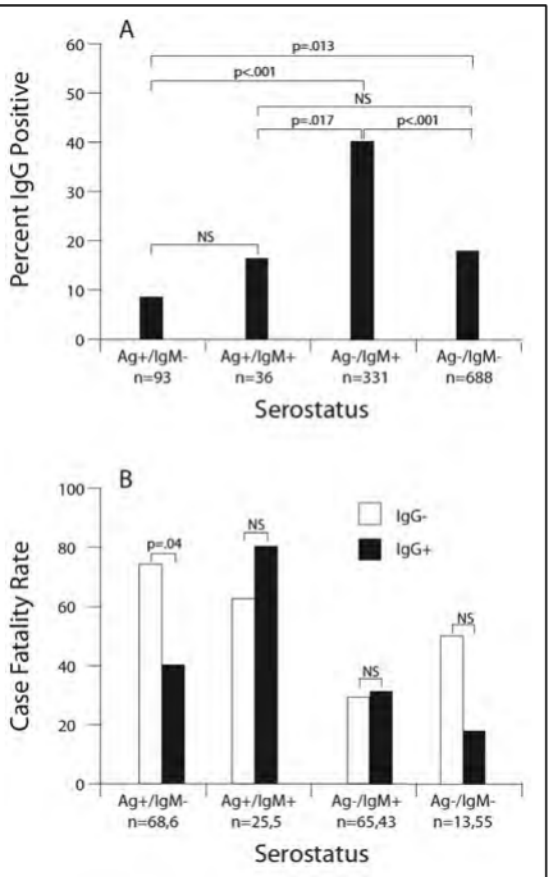


Fig. 69. Anti-LASV IgG in suspected LF patients presenting to the Kenema Government Hospital, 2008-12. Panel A: Percentage of patients with anti-LASV IgG by serostatus. Panel B: Case fatality rates in patients by LASV antigen,anti-LASV IgM, and anti-LASV IgG serostatus. Logistic regression models predicting IgG-positivity and case fatality rates were used to carry out the within and between group comparisons . NS = not significant.

The overall CFR in suspected LF cases with LASV antigenemia was 69.1% (103/149 patients died). The CFR was similar in Ag+ cases with or without anti-LASV IgM, 63.6% (28/44) and 7 1.4% (75/105), respectively (Fig. 57). In patients without LASV antigenemia but with evidence of prior LASV exposure (Ag-/IgM+), the CFR was 29.3% (46/157) (Fig. 68). An analysis of data from Ag-/IgM+ patients for which more than one IgM test result was available indicated that most (70/83, 84.3%) did not show a significant increase in anti-LASV IgM levels during their stay in the Lassa Ward. It is likely that these patients, many of whom were febrile, had an illness other than LF. There was only one fatality among Ag-/IgM+ patients (1/13, 7.7%) who were observed to have a rising anti-LASV IgM level over the course of their stay in the Lassa Ward. These patients appeared to have recently controlled their LASV viremia, generally had only moderately elevated aspartate aminotransferase levels (data not shown), which is an indicator or severity in LF, and were considered to be in the early stages of convalescence. IgM has shown to persist for months or years in convalescent patients. Patients who fit the LF case definition but who tested Ag-/IgM- were considered to not have LF. The CFR in this group was 35.4% (64/181) (Fig. 68). We have the calculated CFRs based only on patients for whom we could verify outcomes based on patient records. The CFRs of 29% and 35% in the Ag-/IgM+ and Ag-/IgM- serogroups are likely higher in the subset of patients with verifiable outcomes than the CFRs in these serogroups overall, as only the most severely ill subjects in these groups were admitted to the Lassa Ward.

The prevalence of antiLASV IgG was significantly higher in Ag-/IgM+ patients, than in Ag-/IgM- patients and patients in other serogroups, which is consistent with inhibition of antibody class-switching (Fig. 69). Among subjects presenting with an UAFI, as determined by the absence of LASV Ag or anti-LASV IgM, 125/688 (18.2%) had anti-LASV IgG indicating prior exposure to LASV. Only six patients (two fatal) showed an Ag+/IgM-/IgG+ profile, which is consistent with a secondary LASV infection. The case fatality rate in these patients was significantly lower (p=.04) than in Ag+/IgM-/IgG- patients with primary LF (Fig. 69). These results confirm prior evidence suggesting that secondary infection with LASV is possible, but results in milder disease than primary LASV infection.[48]

D. EPITOPE SUBMISSION

Milestone 10: As specified in the RFP, submit information on LASV B cell epitopes developed under this contract to the Immune Epitope Database and Analysis Resource (www.ImmuneEpitope.org) to facilitate access and use of these data and tools by the broader research community.

The collaborative team has submitted information on LASV B cell epitopes to the Immune Epitope Database and Analysis Resource (www.ImmuneEpitope.org) to facilitate access and use of these data and tools by the broader research community.

Goals:

- Compile and analyze data regarding B cell epitopes of LASV
- Submit 6-12 LASV GP, NP and Z B cell epitopes to ImmuneEpitope.org

OVERVIEW OF CONTRACT DELIVERABLES ACHIEVED IN MILESTONE 10.

We will exceed Milestone 10 contract goals.

- As described in this instant report we have compiled and analyzed data regarding B cell epitopes of LASV.
- As directed by the advisory committee the focus has been on epitopes of the LASV glycoprotein. The LASV 19 GP epitopes in Table 6 have already been submitted to the IEDB, which exceeds the contract deliverable goal of 6-12. We will submit data on approximately 100 more monoclonal antibody-defined GP epitopes, as well as 6-12 epitopes each on NP and Z.

Table 6. Epitopes submitted to IEDB.

Group	huMAb	Neutralizing	Epitope type	Epitope (amino acids)
GP1-A	19.7E	+	conformational	111-117
GP1-B	21.6 G	-	conformational	121-133
GP1-B	2.4F	-	conformational	122-133
GP1-C	3.3B	-	conformational	126-133
GP1-C	5.6H	-	conformational	126-133
GP1-BC	18.5D	-	conformational	121-133
GP1-BC	39.3G	-	conformational	121-133
GP1-BC	37.4E	-	conformational	121-133
GP2-A1	37.2D	+	conformational	270-278
GP2-A1	25.10C	+	conformational	270-278
GP2-A1	36.1F	+	conformational	270-278
GP2-A2	37.7H	+	conformational	364-370; 377-388
GP2-A2	25.6A	+	conformational	364-370; 377-388
GP2-A2	36.9F	+	conformational	364-370; 377-388
GP2-A2	2.9D	+	conformational	364-370; 377-388
GP2-B	22.5D	-	linear	300-315
GP2-B	4.1F	-	linear	303-309
GP2-B	8.8B	-	linear	303-309
GP2-C	37.2G	+	conformational	350-360

Update about meetings/site visits

All meetings, site visits and teleconferences were held as scheduled.

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Grant Number: 1U01AI082119-01

Principal Investigator(s):
ROBERT F GARRY, PHD

Project Title: Preclinical development of recombinant antigen diagnostics for Lassa fever

DIRECTOR, RESEARCH ADMIN
TULANE UNIV HLTH SCIENCES CTR
1430 TULANE AVENUE
NEW ORLEANS, LA 70112

Award e-mailed to: elecnotf@tulane.edu

Budget Period: 04/22/2009 – 03/31/2010
Project Period: 04/22/2009 – 03/31/2014

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $1,499,626 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to TULANE UNIVERSITY OF LOUISIANA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 31 USC 6305 & 6306 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release or other document that cites results from NIH grant-supported research must include an acknowledgment of NIH grant support and disclaimer such as "The project described was supported by Award Number U01AI082119 from the National Institute Of Allergy And Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health."

Award recipients are required to comply with the NIH Public Access Policy. This includes submission to PubMed Central (PMC), upon acceptance for publication, an electronic version of a final peer-reviewed, manuscript resulting from research supported in whole or in part, with direct costs from National Institutes of Health. The author's final peer-reviewed manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process. For additional information, please visit http://publicaccess.nih.gov/.

Award recipients must promote objectivity in research by establishing standards to ensure that the design, conduct and reporting of research funded under NIH-funded awards are not biased by a conflicting financial interest of an Investigator. Investigator is defined as the Principal Investigator and any other person who is responsible for the design, conduct, or reporting of NIH-funded research or proposed research, including the Investigator's spouse and dependent children. Awardees must have a written administrative process to identify and manage financial conflict of interest and must inform Investigators of the conflict of interest policy and of the Investigators' responsibilities. Prior to expenditure of these awarded funds, the Awardee must report to the NIH Awarding Component the existence of a conflicting interest and within 60 days of any new conflicting interests identified after the initial report. Awardees must comply with these and all other aspects of 42 CFR Part 50, Subpart F. These requirements also apply to subgrantees, contractors, or collaborators engaged by the Awardee under this award. The NIH website http://grants.nih.gov/grants/policy/coi/index.htm provides additional information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Leslie D. Boggs
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 1U01AI082119-01

Award Calculation (U.S. Dollars)

Salaries and Wages	$210,766
Fringe Benefits	$40,885
Equipment	$50,000
Supplies	$50,000
Travel Costs	$20,000
Other Costs	$90,000
Consortium/Contractual Cost	$824,016
Federal Direct Costs	$1,285,667
Federal F&A Costs	$213,959
Approved Budget	$1,499,626
Federal Share	$1,499,626
TOTAL FEDERAL AWARD AMOUNT	$1,499,626
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$1,499,626

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$1,499,626	$1,499,626
2	$1,367,589	$1,367,589
3	$1,372,918	$1,372,918
4	$1,401,819	$1,401,819
5	$1,431,586	$1,431,586

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1720423889A1
Document Number:	UAI082119A
Fiscal Year:	2009

IC	*CAN*	*2009*	*2010*	*2011*	*2012*	*2013*
AI	8472315	$1,499,626	$1,367,589	$1,372,918	$1,401,819	$1,431,586

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M32B B / **OC**: 414L / **Processed**: eRA Commons User Name 04/14/2009

SECTION II – PAYMENT/HOTLINE INFORMATION – 1U01AI082119-01

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1U01AI082119-01

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

Carry over of an unobligated balance into the next budget period requires Grants Management Officer prior approval.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 1U01AI082119-01

The budget period anniversary start date for future year(s) will be April 1.
****

This award is subject to the Terms and Conditions of Award as set forth in the SPECIAL REQUIREMENTS section of RFA-AI-08-001, NIH Guide to Grants and Contracts, 02/21/2008. These special terms and conditions are incorporated in this award by reference.

Copies of the RFA may be accessed at the following Internet address:
<http://www.nih.gov/grants/guide/index.html>

As mandated in this RFA, the principal investigator should submit a performance plan to the NIAID program official that details specific milestones and timelines for achieving each milestone. Milestones should be linked to the annual funding cycle and submission of the annual progress report. The plan should include the specific criteria to be used in evaluating the degree of progress made in achieving each milestone.

The timelines and milestones must be approved by the Program Official within 30 days from the issue date of award.

Such timelines and milestones shall be agreed upon by the principal investigator and the NIAID program official before funds may be released.

To receive consideration for funding of each successive year, the annual progress report and an Updated Product Development Plan must be received two months prior to the end of the current funding period, demonstrating that the milestones defined for that funding year have been met.

The award may be adjusted in time or funding, as necessary, if the grantee fails to meet the agreed upon milestones.
****

This award includes funds awarded for consortium activity with Autoimmune Technologies, LLC in the amount of $282,866.
This award includes funds awarded for consortium activity with Corgenix Medical Corporation in the amount of $283,094.
This award includes funds awarded for consortium activity with Vybion in the amount of $258,056 ($122,884 direct costs + $135,172 facilities and administrative costs).

Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants1.nih.gov/grants/policy/nihgps_2003/NIHGPS_Part12.htm#_Toc54600251, pages 224-227.
****

Total direct funds (salary, fringe benefits and tuition remission) for the Graduate Student are provided at the NIH maximum allowable amount ($37,368), stipend level zero of the Ruth L. Kirschstein National Research Service Award. Please refer to the NIH Guide for Grants and Contracts, Notice: OD-09-075, dated March 27,2009. See http://grants1.nih.gov/grants/guide/notice-files/NOT-OD-09-075.html

for more information. Support recommended for future years has been adjusted accordingly, if applicable.
****

None of the funds in this award shall be used to pay the salary of an individual at a rate per year in excess of the amounts reflected in the following NIH Guide Notice: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-09-037.html Therefore, this award and/or future years are adjusted accordingly, if applicable.
****

The research proposed in this grant may involve Select Agents and/or Highly Pathogenic Agents. NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that, under some circumstances, may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm), your Institutional Biosafety Committee (IBC) or equivalent body, or appropriate designated institutional biosafety official. If there is ambiguity in the BMBL guidelines and/or there is disagreement among the BMBL, an institutional committee or institutional official, the highest recommended containment level must be used.

When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If the work involves Select Agents and/or Highly Pathogenic Agents. Also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s);
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

For domestic work with Select Agents provide documentation of Registration status of all domestic organizations/entities where Select Agent(s) will be used

Please be advised that changes in the use of a Select Agent will likely be considered a change in scope and, therefore, require NIH awarding office prior approval.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Christy S. Leake
Email: cleake@mail.nih.gov **Phone**: 301-402-5937 **Fax**: 301-493-0597

Program Official: Patricia M. Repik
Email: prepik@niaid.nih.gov **Phone**: 301-451-3504 **Fax**: 301-480-1594

SPREADSHEET SUMMARY
GRANT NUMBER: 1U01AI082119-01

Budget	***Year 1***	***Year 2***	***Year 3***	***Year 4***	***Year 5***
Salaries and Wages	$210,766	$217,089	$223,602	$230,310	$237,219
Fringe Benefits	$40,885	$42,112	$43,375	$44,676	$46,017
Equipment	$50,000				
Supplies	$50,000	$50,000	$50,000	$50,000	$50,000
Travel Costs	$20,000	$20,000	$20,000	$20,000	$20,000
Other Costs	$90,000	$90,000	$90,000	$90,000	$90,000
Consortium/Contractual Cost	$824,016	$767,412	$761,153	$778,117	$795,591
TOTAL FEDERAL DC	$1,285,667	$1,186,613	$1,188,130	$1,213,103	$1,238,827
TOTAL FEDERAL F&A	$213,959	$180,976	$184,788	$188,716	$192,759
TOTAL COST	$1,499,626	$1,367,589	$1,372,918	$1,401,819	$1,431,586

Facilities and Administrative Costs	***Year 1***	***Year 2***	***Year 3***	***Year 4***	***Year 5***
F&A Cost Rate 1	49%	49%	49%	49%	49%
F&A Cost Base 1	$436,651	$369,339	$377,118	$385,135	$393,386
F&A Costs 1	$213,959	$180,976	$184,788	$188,716	$192,759

Form Approved Through 11/30/2010 OMB No. 0925-0001

Department of Health and Human Services
National Institutes of Health

12144857

Application JUN 19 2008

Do not exceed character length restrictions indicated.

PI: **GARRY, ROBERT F** Council: 01/2009

1 U01 AI082119-01 IPF:8424601

Dual:

IRG: ZAI1 SRC(99) Received: 06/19/2008

1. TITLE OF PROJECT *(Do not exceed 81 characters, including spaces a...*

Preclinical development of recombinant antigen diagnostics for Lassa fever

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ☒ YES *(If "Yes," state number and title)*

Number: RFA-AI-08-001 Title: Cooperative Research Partnerships for Biodefense and Emerging Infectious Diseases (U01)

3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR | **New Investigator** ☒ **No** ☐ **Yes**

3a. NAME (Last, first, middle): Garry, Robert, F.

3b. DEGREE(S): B.S. Ph.D.

3h. eRA Commons User Name: eRA Commons User Name

3c. POSITION TITLE: Professor

3d. MAILING ADDRESS *(Street, city, state, zip code)*: 1430 Tulane Avenue, New Orleans, Louisiana 70112

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT: Microbiology and Immunology

3f. MAJOR SUBDIVISION: Medicine

3g. TELEPHONE AND FAX *(Area code, number and extension)*: TEL: 504-98-2027 FAX: 504-988-1994

E-MAIL ADDRESS: rfgarry@tulane.edu

4. HUMAN SUBJECTS RESEARCH ☐ No ☒ Yes | 4a. Research Exempt ☒ No ☐ Yes | If "Yes," Exemption No.

4b. Federal-Wide Assurance No.: FWA00002055

4c. Clinical Trial ☒ No ☐ Yes

4d. NIH-defined Phase III Clinical Trial ☒ No ☐ Yes

5. VERTEBRATE ANIMALS ☒ No ☐ Yes | 5a. Animal Welfare Assurance No.

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
06/01/2009	05/31/14	$1,929,363	$2,162,318	$8,910,711	$9,913,636

9. APPLICANT ORGANIZATION

Name: Tulane University Health Sciences Center

Address: 1430 Tulane Avenue, New Orleans, Louisiana 70112

10. TYPE OF ORGANIZATION

Public: → ☐ Federal ☐ State ☐ Local

Private: → ☒ Private Nonprofit

For-profit: → ☐ General ☐ Small Business

☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER: 72-0423889

DUNS NO. 053785812 Cong. District 02

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE

Name: Kathleen M. Kozar

Title: Director, Research Administration

Address: 1430 Tulane Avenue, New Orleans, Louisiana 70112

Tel: 504-988-5613 FAX: 504-988-1748

E-Mail: elecnotf@tulane.edu

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION

Name: Kathleen M. Kozar

Title: Director, Research Administration

Address: 1430 Tulane Avenue, New Orleans, Louisiana 70112

Tel: 504-988-5613 FAX: 504-988-1748

E-Mail: kkozar@tulane.edu

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)* Kathleen M Kozar DATE 6/18/08

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert, F.

PROJECT SUMMARY (See instructions):

This project will develop **Diagnostics For Biodefense** against Lassa fever, a severe, often fatal viral hemorrhagic fever (VHF). Because of its high case fatality rate, ability to spread easily by human-human contact, and potential for aerosol release, Lassa virus (LASV), the causative agent of Lassa fever, is classified as a Biosafety Level 4 and NIAID Biodefense category A agent. Our team has successfully produced prototype LASV enzyme-linked immunosorbent assays (ELISA) that are based on recombinant proteins rather than on reagents that must be produced in high containment laboratories. We have also newly established a research program in Sierra Leone, an area endemic for LASV, that provides unique clinical and laboratory resources for VHF research. We will now perform critical steps in the preclinical development of commercial recombinant antigen Lassa fever diagnostics. In MILESTONE 1 we will development commercial Lassa fever recombinant antigen-capture and immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody-capture ELISA using a Performance Panel of well-characterized sera. In MILESTONE 2 we will development Lassa fever recombinant lateral flow point-of-care diagnostics. In MILESTONE 3 we will convert to manufacturing recombinant ELISA and lateral flow assays under Good Manufacturing Practices (GMP) with Quality Assurance (QA)/Quality Control (QC) to provide quantities of commercial grade diagnostic kits sufficient for preclinical evaluation of design control parameters to achieve benchmarks required for FDA approval. In MILESTONE 4 we will optimize scale up/purification of recombinant LASV proteins, GP1, GP2, and NP and monoclonal antibodies (mAbs) to recombinant LASV GP1, GP2, and NP. We will also convert to manufacturing with QA/QC, to provide quantities of recombinant proteins and mAbs sufficient for development and testing of commercial assays. In MILESTONE 5 we will define and collect positive and negative sera for assay validation from diverse regions across the Lassa fever endemic range of West Africa and elsewhere. These sera will be used to test and compare the commercial LASV recombinant ELISA and recombinant lateral flow point-of-care diagnostic assays with results from BSL-4 ELISA and PCR assays.

RELEVANCE (See instructions):

The potential use of LASV as a biological weapon necessitates development of methods to diagnose individuals exposed to and/or infected with LASV. The impact of Lassa fever in endemic areas of West Africa is immense, and a safe and effective diagnostic can also provide a very significant public health benefit.

PROJECT/PERFORMANCE SITE(S) (If additional space is needed, use Project/Performance Site Format Page)

Project/Performance Site Primary Location			
Organizational Name: Tulane University Health Sciences Center			
DUNS: 053785812			
Street 1: 1430 Tulane Avenue	Street 2:		
City: New Orleans	County: Orleans Parish	State: Louisiana	
Province: NA	Country: United States	Zip/Postal Code: 70112	
Project/Performance Site Congressional Districts: 02			

Additional Project/Performance Site Location			
Organizational Name: Corgenix Medical Corporation			
DUNS: 619834542			
Street 1: 11575 Main Street	Street 2: Suite 400		
City: Broomfield	County: Broomfield	State: Colorado	
Province: NA	Country: United States	Zip/Postal Code: 80020	
Project/Performance Site Congressional Districts:			

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert, F.

Use only if additional space is needed to list additional project/performance sites.

Additional Project/Performance Site Location		
Organizational Name: Autoimmune Technologies, LLC		
DUNS: 946839156		
Street 1: 1010 Commons Street	Street 2: Suite 1705	
City: New Orleans	County: Orleans Parish	State: Louisiana
Province: NA	Country: United States	Zip/Postal Code: 70118
Project/Performance Site Congressional Districts: 02		

Additional Project/Performance Site Location		
Organizational Name: Vybion		
DUNS: 161463551		
Street 1: 33 Thornwood Drive	Street 2:	
City: Ithica	County: Tompkins	State: New York
Province: NA	Country: United States	Zip/Postal Code: 14850
Project/Performance Site Congressional Districts: 24		

Additional Project/Performance Site Location		
Organizational Name: United States Army Institute for Infectious Diseases		
DUNS: NA		
Street 1: 1425 Porter Street	Street 2: Fort Detrick	
City: Frederick	County: Frederick	State: Maryland
Province: NA	Country: United States	Zip/Postal Code: 21702
Project/Performance Site Congressional Districts: 06		

Additional Project/Performance Site Location		
Organizational Name:		
DUNS:		
Street 1:	Street 2:	
City:	County:	State:
Province:	Country:	Zip/Postal Code:
Project/Performance Site Congressional Districts:		

Additional Project/Performance Site Location		
Organizational Name:		
DUNS:		
Street 1:	Street 2:	
City:	County:	State:
Province:	Country:	Zip/Postal Code:
Project/Performance Site Congressional Districts:		

SENIOR/KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Program Director(s)/Principal Investigator(s). List all other senior/key personnel in alphabetical order, last name first.

Name	eRA Commons User Name	Organization	Role on Project
Robert F. Garry, Ph.D.	eRA Commons User Name	TUHSC	Principal Investigator
Daniel G. Bausch, M.D.		TSPHTM	Investigator
Luis M. Branco		Autoimmune	Investigator
Joseph N. Fair, Ph.D.		Southern Research Inst.	Consultant
Wallace R. Fish, Ph.D.		Vybion	Investigator
Mary C. Guttieri, Ph.D.		USAMRIID	Director, subcontract
F. Jon Geske, Ph.D.		Corgenix	Investigator
John D. Noti, Ph.D.		Vybion	Director, subcontract
Gregory W. Svanas, Ph.D.		Corgenix	Director, subcontract
Russell B. Wilson, Ph.D.		Autoimmune	Director, subcontract

OTHER SIGNIFICANT CONTRIBUTORS

Name	Organization	Role on Project
George O. Akpede, FWACP	Irrua Hospital, Nigeria	Collaborator
Mamadou Coulibaly	Tulane-CIRIT, N'Zérékoré, Guinea	Collaborator
Jerry Dubai	Ministry of Health, Monrovia, Liberia	Collaborator
Deli Enria, MD	INEV, Pergamino, Argentina	Collaborator
Augustine Goba	Lassa Lab, KGH, Sierra Leone	Collaborator
Humarr Khan, MD	Lassa Ward, KGH, Sierra Leone	Collaborator
Clarence J. Peters, MD	UTMB, Galveston, Texas	Collaborator
Robert Swanepoel, PhD	NICD, Sandringham, South Africa	Collaborator

maman Embryonic Stem Cells ☐ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. *Use continuation pages as needed.*

If a specific line cannot be referenced at this time, include a statement that one from the Registry will be used.

The name of the program director/principal investigator must be provided at the top of each printed page and each continuation page.

RESEARCH GRANT

TABLE OF CONTENTS

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert, F.

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY - TUHSC

FROM 06/01/09 THROUGH 05/31/10

PERSONNEL (Applicant organization only) NAME	ROLE ON PROJECT	Months Devoted to Project: Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	DOLLAR AMOUNT REQUESTED (omit cents): SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Robert F. Garry, PhD	PD/PI	EFFORT			Institutional Base Salary	39,750	7,791	47,541
Daniel G. Bausch, MD	Investgator					36,331	7,121	43,452
Allyson Haislip	Laboratory Manager					14,435	3,796	18,231
TBN	Program Coordinator					35,000	9,205	44,205
TBN	Technician					35,000	9,205	44,205
Personal Info	Graduate Student					25,750	2,034	27,784
SUBTOTALS →						186,266	39,152	225,418

CONSULTANT COSTS Joseph N. Fair, Ph.D.	0
EQUIPMENT *(Itemize)* -20° and -80° freezer, solar panels.	50,000
SUPPLIES *(Itemize by category)* Molecular biology reagents and supply for assay development and testing.	50,000
TRAVEL Travel to West Africa, and a required yearly meeting with NIAID Program Staff.	20,000
PATIENT CARE COSTS — INPATIENT	40,000
PATIENT CARE COSTS — OUTPATIENT	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	0
OTHER EXPENSES *(Itemize by category)* Supplies ad other expenses related to field collection of serum samples.	40,000
CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS	1,234,437
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*	$ 1,659,855
CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATIVE COSTS	269,508
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD	$ 1,929,363

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY - TUHSC

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD (from Form Page 4)	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		225,418	232,182	239,151	246,323	253,712
CONSULTANT COSTS		0	0	0	0	0
EQUIPMENT		50,000	00	0	0	0
SUPPLIES		50,000	50,000	50,000	50,000	50,000
TRAVEL		20,000	20,000	20,000	20,000	20,000
PATIENT CARE COSTS	INPATIENT	40,000	40,000	40,000	40,000	40,000
	OUTPATIENT	0	0	0	0	0
ALTERATIONS AND RENOVATIONS		0	0	0	0	0
OTHER EXPENSES		40,000	40,000	40,000	40,000	40,000
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	1,234,437	1,076,824	1,090,299	1,113,087	1,331,342
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*		1,659,855	1,459,006	1,479,450	1,509,410	1,535,054
CONSORTIUM/ CONTRACTUAL COSTS	F&A	269,508	266,959	237,868	243,765	249,836
TOTAL DIRECT COSTS		1,929,363	1,725,965	1,717,318	1,753,175	1,784,890

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 8,910,711

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

See continuation pages.

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert F.

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY – Autoimmune Technologies

FROM: 06-01-09 THROUGH: 05-31-10

PERSONNEL (Applicant organization only)		Months Devoted to Project				DOLLAR AMOUNT REQUESTED (omit cents)		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Russell B. Wilson	Director, subcontract	EFFORT			Institutional Base Salary	15,000	3,750	18,750
Luis M. Branco	Investigator					115,000	28,750	143,750
Erin M. O'Neill	Laboratory Technician II					38,000	9,500	47,500
SUBTOTALS →						168,000	42,000	210,000

CONSULTANT COSTS		
EQUIPMENT *(Itemize)* Cell culture and bacterial fermentation Benchtop bioreactor BF-110 (10L)		48,132
SUPPLIES *(Itemize by category)* Bacterial fermentation and mammalian cell culture supplies. Appropriate resins, dedicated columns, and accessories for protein purification.		80,000
TRAVEL Funds are requested to support travel of Dr. Wilson and Mr. Blanco to the facilities of Vybion or Corgenix for review and coordination of efforts.		4,000
PATIENT CARE COSTS	INPATIENT	0
	OUTPATIENT	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		0
OTHER EXPENSES *(Itemize by category)*		0
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	0
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 342,132
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	29,400
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 371,532

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY – Autoimmune Technologies

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD (from Form Page 4)	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		210,000	216,308	222,798	229,480	236,365
CONSULTANT COSTS		0	0	0	0	0
EQUIPMENT		48,132	0	0	0	0
SUPPLIES		80,000	80,000	80,000	80,000	80,000
TRAVEL		4,000	4,000	4,000	4,000	4,000
PATIENT CARE COSTS	INPATIENT	0	0	0	0	0
	OUTPATIENT	0	0	0	0	0
ALTERATIONS AND RENOVATIONS		0	0	0	0	0
OTHER EXPENSES		0	0	0	0	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	0	0	0	0	0
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*		342,132	300,308	306,798	313,480	320365
CONSORTIUM/ CONTRACTUAL COSTS	F&A	29,400	30,031	30,680	31,348	32037
TOTAL DIRECT COSTS		371,532	330,339	337,478	344,828	352,402
TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD						$ 1,736,579

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

See continuation pages.

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert F.

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY – Corgenix

FROM 06/01/09 THROUGH 05/31/10

PERSONNEL (Applicant organization only)		Months Devoted to Project				DOLLAR AMOUNT REQUESTED (omit cents)		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Gregory Svanas, Ph.D.	Director, subcontract	EFFORT			Institutional Base Salary	65,600	16,400	82,000
F. Jon Geske, Ph.D.	Investigator					41,000	10,250	51,250
Luis Lopez, M.D., Ph.D.	Investigator					47,825	11,956	59,781
Matt Boisen	R&D staff					31,000	7,750	38,750
Ivana Muncey	R&D staff					31,200	7,800	39,000
Dan Simpson	Regulatory affairs					8,250	2,063	10,313
SUBTOTALS →						224,875	56,219	281,094

CONSULTANT COSTS		15,000
EQUIPMENT *(Itemize)* Centrifuge, BioDot XYZ Dispending System, Benchtop centrifuge, freezer, miscellaneous equipment.		140,000
SUPPLIES *(Itemize by category)* Supplies both for ELISA microplate development and lateral flow (rapid) product development.		40,000
TRAVEL Bi-annual meetings with Tulane and other development partners to discuss progress and future planning aspects of the project. Scientific meetings on assay development.		12,000
PATIENT CARE COSTS	INPATIENT	0
	OUTPATIENT	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		0
OTHER EXPENSES *(Itemize by category)*		0
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	0
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 488,094
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	0
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 488,094

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY – Corgenix

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD (from Form Page 4)	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		281,094	301,316	308,562	316,025	323,713
CONSULTANT COSTS		15,000	15,000	15,000	15,000	15,000
EQUIPMENT		140,000	0	0	0	0
SUPPLIES		40,000	40,000	60,000	60,000	60,000
TRAVEL		12,000	12,000	12,000	12,000	12,000
PATIENT CARE COSTS	INPATIENT	0	0	0	0	0
	OUTPATIENT	0	0	0	0	0
ALTERATIONS AND RENOVATIONS		0	0	0	0	0
OTHER EXPENSES		0	0	0	0	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	0	0	0	0	0
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*		488,094	368,316	395,562	403,025	410,713
CONSORTIUM/ CONTRACTUAL COSTS	F&A	0	0	0	0	0
TOTAL DIRECT COSTS		488,094	368,316	395,562	403,025	410,713
TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD						**$** 2,065,710

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

See continuation pages.

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert F.

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY - Vybion

FROM 06/01/09 THROUGH 05/31/10

PERSONNEL (Applicant organization only)		Months Devoted to Project				DOLLAR AMOUNT REQUESTED (omit cents)		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
John D. Noti, Ph.D.	Director, subcontract	EFFORT			Institutional Base Salary	5,000	1,450	6,450
Wallace R. Fish, Ph.D.	Investigator					37,500	10,875	48,375
Hui Zhu	Fermentation Scientist					31,500	9,135	40,635
Scot Fletcher	Purification Scientist					58,000	16,820	74,820
SUBTOTALS →						132,000	38,280	170,280

CONSULTANT COSTS		0
EQUIPMENT *(Itemize)*		0
SUPPLIES *(Itemize by category)* Fermentation and mammalian cell culture supplies.		48,000
TRAVEL		0
PATIENT CARE COSTS	INPATIENT	0
	OUTPATIENT	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		0
OTHER EXPENSES *(Itemize by category)*		0
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	0
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7e, Face Page)*		$ 218,280
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	240,108
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 458,388

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY - Vybion

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD (from Form Page 4)	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		170,280	175,389	158,353	163,106	167,999
CONSULTANT COSTS		0	0	0	0	0
EQUIPMENT		0	0	0	0	0
SUPPLIES		48,000	40,000	30,000	30,000	30,000
TRAVEL		0	0	0	0	0
PATIENT CARE COSTS	INPATIENT	0	0	0	0	0
	OUTPATIENT	0	0	0	0	0
ALTERATIONS AND RENOVATIONS		0	0	0	0	0
OTHER EXPENSES		0	0	0	0	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	0	0	0	0	0
SUBTOTAL DIRECT COSTS *(Sum = Item 8e, Face Page)*		218,280	215,389	188,353	193,106	197,999
CONSORTIUM/ CONTRACTUAL COSTS	F&A	240,108	236,928	207,188	212,417	217,799
TOTAL DIRECT COSTS		458,388	452,317	395,541	405,523	415,798

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD **$** 2,127,567

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

See continuation pages.

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert F.

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY - USAMRIID

FROM 06/01/09 THROUGH 05/31/10

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Mary C. Guttieri	Director, subcontracto	EFFORT			-	0	0	0
Government contractor	Research Associate				Institutional Base Salary	100,000	22,000	122,000
SUBTOTALS →						100,000	22,000	122,000

CONSULTANT COSTS		0
EQUIPMENT *(Itemize)*		0
SUPPLIES *(Itemize by category)* Cell culture, PCR and molecular biology reagents.		3,500
TRAVEL Travel to Lassa Fever Laboratory in Kenema, Sierra Leone, Tulane or scientific conferences		15,000
PATIENT CARE COSTS	INPATIENT	0
	OUTPATIENT	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		0
OTHER EXPENSES *(Itemize by category)* Transportation: transfer of reagents to extramural collaborators ($2,000) Cell Culture Support Services ($3,054.17) Special Immunizations (SIP): Fees for health maintenance oversight required for government contractor to work in biosafety level-4 laboratory ($8,189) Visual Information Support Services ($888) Facility Infrastructure Support ($31,300/FTE)		45,431
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	0
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 185,931
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	0
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 185,931

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY - USAMRIID

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	122,000	126,880	131,955	137,233	142,722
CONSULTANT COSTS	0	0	0	0	0
EQUIPMENT	0	0	0	0	0
SUPPLIES	3,500	3,500	3,500	2,500	2,000
TRAVEL	15,000	15,000	15,000	12,000	5,000
PATIENT CARE COSTS	0	0	0	0	0
	0	0	0	0	0
ALTERATIONS AND RENOVATIONS	0	0	0	0	0
OTHER EXPENSES	45,431	47,431	49,131	50,743	52,543
CONSORTIUM/ CONTRACTUAL COSTS	0	0	0	0	0
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	185,931	192,811	199,586	202,476	202,265
CONSORTIUM/ CONTRACTUAL COSTS	0	0	0	0	0
TOTAL DIRECT COSTS	185,931	192,811	199,586	202,476	202,265

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD **$** 983,069

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

See continuation pages.

BUDGET JUSTIFICATION

Overview of personal and resource utilization

Tulane University Health Sciences Center (TUHSC)

- Dr. Garry will serve as PI and direct the overall research program.
- Dr. Bausch will oversee the acquisition of clinical samples in Sierra Leone, Guinea, Liberia, Nigeria, Argentina and elsewhere.
- Tulane scientists will maintain clinical and laboratory infrastructure at the Lassa Ward/Laboratory of Kenema Government Hospital, Kenema, Sierra Leone.
- Tulane scientists will perform iterative field-testing of various commercial prototypes LASV and lateral flow assays under development throughout the project period.
- LASV-specific mAb will be characterized by Tulane scientists to enable selection of mAb with diverse specificities and binding characteristics.
- Tulane scientists will compare the recombinant antigen based Lassa fever ELISA and lateral flow assays under development with existing BSL-4 diagnostic assays and PCR.
- Tulane scientists will compile the preclinical and field-testing data that will be used for eventual regulatory submission of each of the ELISA and lateral flow kits.

Autoimmune Technologies, LLC (AIT)

- AIT will continue to provide recombinant LASV NP, GP1 and GP2 produced in *E. coli* Rosetta cells to Corgenix for the optimization, production and field-testing of recombinant Lassa fever ELISA and lateral flow assays.
- Hybridoma scale-up, mAb purification and production of LASV-specific Mobs will be performed by AIT, and these mAbs will be provided to Corgenix for assay production.
- AIT will optimize current methods for production of recombinant LASV NP, GP1 and GP2 in Rosetta strains of *E. coli*.
- AIT will optimize expression LASV GP1 and GP2 in mammalian cell lines.
- AIT will identify optimal purification parameters for LASV NP, GP1 and GP2 from *E. coli* Rosetta and mammalian cells
- Throughout the project, AIT will coordinate and review Consortium activities to ensure compliance with regulatory requirements.

Vybion

- Vybion will optimize expression of LASV NP, GP1 and GP2 using either: 1) synthetic gene replacement in *E. coli*, 2) targeting to inclusion bodies in *E. coli* followed by protein refolding or 3) expression following synthetic gene replacement in *Pichia pastoris*.
- Vybion in cooperation with AIT will define parameters for up-scale production of LASV NP, GP1 and GP2.
- Vybion will identify optimal purification parameters for LASV NP, GP1 and GP2 from alternative expression systems.
- Vybion will provide Corgenix scientists with recombinant LASV NP, GP1 and GP2 produced by optimum procedures for commercial recombinant Lassa fever ELISA and lateral flow assays.

Corgenix, Inc.

- Using the recombinant proteins and murine mAb reagents, commercializable Ag-capture, IgM- and IgG-capture Lassa fever ELISA will be developed and optimized in cooperation with Tulane and AIT scientists.
- Using the recombinant proteins and murine mAb reagents, commercializable Ag-capture, IgM- and IgG-capture Lassa fever lateral flow assays will be developed and optimized in cooperation with Tulane and AIT scientists.
- Corgenix will produce pilot lots of commercalizable Lassa fever ELISA and lateral flow kits that will be used for validation studies in West Africa and elsewhere.
- Corgenix will perform quality control, standardization, scale-up, performance, reproducibility, and assist in regulatory compliance aspects of the Lassa fever ELISA and lateral flow assays under development.

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)

- Compare the recombinant antigen based Lassa fever diagnostic assays under development with existing BSL-4 Lassa fever diagnostic assays, and PCR.
- USAMRIID scientists will provide irradiated stocks of various arenaviruses necessary to optimize prototype antigen capture assays and test cross-reactivity.
- All live arenavirus culture studies will be performed in the secure BSL-4 laboratories at USAMRIID.
- USAMRIID scientists will compare Lassa fever ELISA with PCR-based assays.
- USAMRIID scientists will provide detailed sequence analysis of LASV isolated from clinical samples obtained in West Africa.

Kenema Government Hospital, Kenema, Sierra Leone

- KGH is a major source of samples from patients with suspected Lassa virus infection. All patients in Sierra Leone with suspected Lassa virus infection are referred to KGM. In-house Lassa testing recombinant assays in various stages of development will be conducted at KGH.

Tulane-International Center for Research on Tropical Infections (Tulane-CIRIT), N'Zérékoré, Guinea

- This laboratory will be another major site for field-testing recombinant Lassa fever assays in various stages of development.

N'Zérékoré Regional Hospital, N'Zérékoré, Guinea

- N'Zérékoré Regional Hospital is a major source of samples from patients with suspected Lassa virus infection.

Faranah Hospital, Faranah, Guinea

- Faranah Hospital is an additional source of samples from Guinean patients with suspected Lassa virus infection.

Instituto National de Enfermedades Virales, Pergamino. Argentina

- INEV is a major source of archival samples from patients with suspected New World virus infection. In-house field-testing of recombinant assays in various stages of development will be conducted at INEV.

Tulane University Health Sciences Center
Budget Justification

Tulane University Health Sciences Center (TUHSC) is academically and administratively affiliated with Tulane University. It is located in downtown New Orleans. TUHSC is comprised of a Medical School, School of Public Health, Hospital and Clinic, Tulane National Primate Research Center and numerous other research centers.

Personnel

Robert F. Garry, Ph.D. will serve as Principal Investigator and devote EFF of his time to the project. Dr. Garry will coordinate the study overall and directly supervise the work of Ms. Haislip and Personal Info He will also work closely with scientists at AIT, Vybion and Corgenix to develop commercial Lassa fever ELISA and lateral flow assays and to produce pilot lots for field-testing. Dr. Garry will be the primary contact with NIH Program Officers for carrying out the studies for this cooperative agreement. He will also be involved in field collection of samples in West Africa, and testing of the ELISA and lateral flow assays in the KGH laboratory in Sierra Leone.

Daniel G. Bausch, M.D, MPH will serve as Investigator and devote EFF of his time to the project. Prior to joining the faculty of Tulane University School of Public Health and Tropical Medicine Dr. Bausch served in the Pathology and Epidemiology Units of the Special Pathogens Branch of the Centers for Disease Control and Prevention (CDC) as Medical Officer and Acting Chief of the Epidemiology Unit. He has extensive International Public Health Experience, including long-time service in Lassa endemic areas of West Africa, and pioneering work on Lassa virus ELISA diagnostic assays that form the basis of this application. His expertise is critical for the success of the proposed study and he will be involved in all aspects. Dr. Bausch will supervise the field collection of samples from symptomatic patients in Africa and control donors. He will directly supervise the field coordinator, and review laboratory infrastructure and operating procedures and supervise revision and upgrades as necessary at each of the cooperating Institutions in West Africa. Dr. Bausch will also coordinate the field collection of sample from persons suspected of having infections with New World arenaviruses with our South American collaborators.

Allyson M. Haislip, B.S. is a full-time Laboratory Supervisor (EFF effort). Ms. Haislip has over twenty years of experience as a research technician, and has devoted most of this time to the study of viruses. Her skills are ideally suited for the proposed study, and she will participate in multiple aspects of the proposed study throughout the project period. Advanced assay development will take place in our stateside laboratories at Tulane University Health Sciences Center, Autoimmune Technologies, Corgenix and USAMRIID. Ms. Haislip and Personal Info will test Lassa virus ELISA and lateral flow assays in various stages of development. Ms. Haislip will also be involved with USAMRIID scientists in cloning and sequence analysis of LASV isolated from clinical samples obtained in West Africa.

Joseph N. Fair, Ph.D. initiated the project on Lassa fever recombinant ELISA while a predoctoral stident in the Garry Laboratory. He will continue to serve as an essential consultant (no salary) as he advances his career in the Biodefense arena.

Personal Info B.S. is a full-time Ph.D. student EFFO effort). She will continue LASV mAb characterization, including epitope mapping to define epitopes that appear to be the most important diagnostically. Personal Info will test Lassa virus ELISA and lateral flow assays in various stages of development. She will also be involved in field collection of samples in West Africa, and testing of the ELISA and lateral flow assays in the KGH laboratory in Sierra Leone.

Program Coordinator EFFO effort). Funds are requested for a full-time program coordinator for the field studies to be conducted in West Africa. This individual will replace the current field coordinator Tiffany Imes, who is continuing studies for a MD degree. The field coordinator will live in Sierra Leone throughout the project period, and coordinate the field studies from the Kenema General Hospital laboratory. A high

degree of coordination will be essential in providing large numbers of quality specimens for testing from patients with Lassa fever, especially considering that several countries in the LASV endemic range are involved. The Program Coordinator will oversee the activities of all the collaborating institutions in Africa, as well as serving as the primary liaison between African collaborators and those in the United States. Specific duties will include implementing and monitoring the process for informed consent, maintaining supplies and databases, organizing shipments of equipment and samples, keeping financial records, and handling logistics for field collection of samples.

Technician EFFO effort). One of the important goals of the proposed project is to compare the recombinant antigen based arenavirus diagnostic assays under development with existing Lassa fever diagnostic assays. Conventional BSL-4 ELISA Lassa fever will be performed on all samples prospectively acquired in Africa at the laboratories of KGH in Kenema, Sierra Leone. This individual will be involved in field collection of samples in Sierra Leone, Guinea, Liberia and Nigeria and testing of the ELISA and lateral flow assays in the KGH laboratory in Sierra Leone, and comparing the performance of these assays in the field with BSL-4 ELISA and PCR.

Equipment
Upgrading of the laboratory and equipment at Kenema Government Hospital (KGH) will be required in order to meet the increased throughput inherent in implementing the current proposal. We are requesting funds to purchase another solar-powered -20 freezer ($8,000). We are also requesting funds to purchase a –80 freezer ($12,000), and to expand the solar panel bank to accommodate an increased overall workload ($30,000).

Supplies
Supplies are requested according to a conservative estimate for molecular characterization of LASV, LASV proteins and the LASV-specific mAb, and for assay development and testing stateside and in our field stations in West Africa.

Travel
Travel to Sierra Leone and Guinea for Drs. Bausch and Garry and the Program Coordinator to Africa for acquisition of samples from LASV infected patients and controls. Funds are also requested for the required travel of the Principal Investigator for an annual meeting in Bethesda to consult with NIAID Program staff.

Clinical
Specimens for Lassa virus diagnostic testing will primarily come from Kenema Government Hospital (KGH) in Sierra Leone. Funds are requested to be spent directly on supplies related to patient care, including intravenous fluids ($3,000 per budget period), ribavirin and other medications ($28,000 per budget period), miscellaneous medical supplies ($5,000 per budget period), personal protective equipment/biosafety upgrades ($4,000 per budget period)

Other Expenses
Funds are requested to be spent directly on supplies related to acquisition and management of the blood specimens to be collected. In addition, to insure optimal management of the patient and the safety of the medical staff patient isolation procedures and facilities will be reviewed for each cooperating Institutions in Sierra Leone, Guinea and South America and revised or up-graded as necessary. These expenses include phlebotomy materials ($3,000 gloves, syringes, needles, vacutainor tubes per budget period), cryovials and cold chain maintenance ($3,000 per budget period), database management/Internet connection and communication ($5,000 per budget period), shipping ($1,500 per budget period), sample acquisition (vehicle maintenance, travel/ temporary lodging: $4,500 per budget period) and laboratory supplies for in-house Lassa fever ELISA, lateral flow and PCR testing and for field testing recombinant assays in various stages of development ($20,000 per budget period). Training sessions in infection control will be conducted, and an adequate supply of personal protective equipment and other necessary

supplies for the optimal care of patients with Lassa fever and nonLassa illnesses, such as syringes and intravenous fluids, will be supplied to each hospital. A portion of the designated funds will go for biosafety training for personnel at all of the aforementioned institutions ($3,000 per budget period).

Autoimmune Technologies - Budget Justification

Autoimmune Technologies, LLC is a biomedical company located in the New Orleans medical technology corridor. The company commercializes proprietary research discoveries of its own and of others, which it acquires through in-licensing. Autoimmune is currently conducting FDA clinical trials of a serum diagnostic test for fibromyalgia and expects to soon make available a virus-based serum diagnostic test for systemic lupus erythematosus.

Personnel

Russell B. Wilson, Ph.D., is President and Chief Science Officer of Autoimmune Technologies, LLC, Dr. Wilson is an experienced cell and molecular biologist and has experience in the expression and isolation of recombinant antigens as well as the development of molecular and immunological based assays. He will be involved in assay development, antigen and antibody purification efforts, and the overall coordination of the commercialization of the assays. He will devote EFF of his effort to this project.

Luis M. Branco, will serve as the Project Manager of Autoimmune's subcontract and will devote EFFO of his time to this project. He will be involved in production of monoclonal and recombinant antigens required to support the assay development activities at Autoimmune and Corgenix. He will also be involved in coordinating the transfer of the process technology transfer to Vybion for the scale up production of the recombinant antigens. Mr. Branco has over 15 years of research and development experience in the fields of molecular biology, immunology, virology, and cell biology particularly in the design and implementation of bacterial, baculoviral, and mammalian expression systems for the production of recombinant therapeutic molecules. Previously, Mr. Branco led the Stable Cell Development Group at Human Genome Sciences, Inc. (HGS) where he developed and implemented innovations for the generation of NS0 and CHO stable cell lines. Prior to HGS, Mr. Branco developed industry experience with a five-year tenure at MedImmune, Inc., where he served important roles in the development of bioassays for MedImmune's flagship product, Synagis™. In addition, he performed leading research in the characterization of MedImmune's leading monoclonal therapeutic, Siplizumab™. Mr. Branco began his career at the University of Massachusetts at Amherst performing post-graduate research in the field of baculovirology, including design of baculovirus expression vectors and insect-cell culture processes.

Erin M. O'Neil will devote EFFO DT percent of her time to this project. She will assist in the development of the screening and prototype LASV and JUNV Ag-capture, IgM and IgG capture ELISA and in the validation studies of the pilot ELISA test kits manufactured by Corgenix. In addition, she will be involved in the production and purification of the monoclonal antibodies and recombinant antigens to support the proposed activities.

Equipment

Cell culture and bacterial fermentation Benchtop bioreactor BF-110 (10L)

The outlined project will require extensive cell culture optimization for up to three to four monoclonal antibodies recognizing each of the recombinant proteins (GP1, GP2, NP). Commonly, high-density cell culture bioreactors runs have a 14-day timeline from inoculation to harvest. It is therefore necessary to have adequate capacity to offset the continuous use of these instruments throughout the project. Including extensive cleaning, sterilization, and sterile hold at the onset, and post harvest cleaning and sterilization each run requires a minimum of 3 full weeks of dedicated bioreactor equipment. Likewise, bacterial fermentation runs will require adequate capacity to supply the required amounts of *E. coli* generated proteins for the project. To produce the required antibodies and recombinant antigens, we are requesting the purchase of a New Brunswick Scientific BioFlo 110 modular 14 L fermentor and bioreactor and an additional 7.5 L cell culture kit for hybridoma cultures.

Supplies

The outlined project will rely heavily of bacterial fermentation and mammalian cell culture based processes. Thus, culture media will constitute a significant portion of the projected supplies budget. Supporting activities require general chemicals, cell culture plasticware, molecular and cell biology reagents, dedicated preparatory glass and plasticware, and required consumables for assay development, formulation and stability studies, and storage of cell lines and protein product lots. Development and implementation of purification methods will require significant amounts of appropriate resins, dedicated columns, and accessories.

Travel

Funds are requested to support travel of Dr. Wilson and Mr. Blanco to the facilities of Vybion or Corgenix for review and coordination of efforts.

Corgenix Budget Justification

Corgenix Medical Corporation, founded in 1990, is engaged in the research, development, manufacture, and marketing of in vitro diagnostic products for use in disease detection and prevention. The company currently sells over 70 diagnostic products on a worldwide basis to hospitals, clinical laboratories, commercial reference laboratories, and research institutions. Corporate headquarters is located in Broomfield, Colorado. Corgenix employs forty individuals at this facility and five in an international marketing office in Peterborough, England. The Company has strategic alliances and other agreements with major biotechnology partners and distributors worldwide. All activities related to this project will be conducted by personnel based in the United States.

Personnel

Gregory W. Svanas, Ph.D., has been Project Director at Corgenix Inc. since March 2006. Dr. Svanas will be responsible for management of the ELISA design process and will devote EFFO of his effort to this project. As the Project Manager, Dr. Svanas will be responsible for the design process for both the microtiter plate and lateral flow formats, managing commercial development of the LASV Ag-capture, IgM and IgG capture assays, coordinating team meetings, facilitating and documenting periodic formal design review and formal approval of project progress.

F. Jon Geske, Ph.D. Senior Scientist EFF effort), will evaluate and standardize the LASV proteins and MAb reagents. He will also be responsible for scale-up production of the Ag-capture, IgM and IgG capture microtiter plate-based ELISAs from prototype assays. He will also coordinate the production of the pilot lots of the LASV Ag-capture, IgM and IgG capture ELISA within the Quality System for testing of the field-collected samples.

Matt Boisen, Scientist, EFF effort), will perform optimization of the lateral flow assays and assist Dr. Svanas in scale-up of lateral flow assay production. Mr. Boisen will coordinate scale-up studies and pilot lot production of pilot lots of the LASV Ag-capture, IgM and IgG capture assays in the lateral flow format for testing of the field-collected samples.

Dan Simpson, Quality Manager, EFFO effort year one increasing to EFFO n years two through five) will coordinate all regulatory and documentation aspects of all LASV antigen- and antibody-capture assays in both the microtiter and lateral flow formats. He will ensure that the documentation of the design and manufacture of the assays will comply with the Corgenix FDA GMP and ISO quality systems.

Ivana Muncy, R&D staff EFF effort first year increasing to EFF in years two through five), will significant technical support for process development. In addition to performing quality control, standardization, scale-up, performance reproducibility, stability studies, Ms. Muncy will assist Mr. Simpson in regulatory compliance aspects of the assays under development.

Luis Lopez, MD, Chief Medical Officer EFF effort), will assist in the design and interpretation of the clinical trials for the LASV assays in both assay formats. He will also be involved in the establishment of diagnostic criteria to be used to calculate the relative sensitivity, specificity, positive predictive value and negative predictive value from the collected data using the lateral flow and the microtiter plate assays.

Consultants

Corgenix will engage an outside consulting firm to assist in all pre-FDA submission activities.

Equipment

Refrigerated SuperSpeed Centrifuge with rotors. Antibody-coated gold solutions are separated from uncoated gold by centrifugation. This separation increases assay sensitivity and reduces nonspecific binding to the immobilized reagents on the lateral flow strip. Centrifugal concentrators will also be used for rapid buffer exchange of small volume reagents and for concentration of uncoated gold solutions. - $40,000

BioDot XYZ Dispensing System. This instrument is used to apply consistent amounts of liquid reagents to the flow membrane and reagent pads for the lateral flow assay format. - $70,000

Refrigerated Multipurpose Benchtop centrifuge. Required for daily handling of small volume chemicals and samples. - $10,000

Ultralow Freezer. Required for sample and antibody storage. $10,000

Miscellaneous equipment. Pipettors and other equipment required for product development. $10,000

Travel

Budgeted travel will be for bi-annual meetings with other representatives of Tulane and other development partners to discuss progress and future planning aspects of the project. Travel will require flights, car rental, lodging and meals at standard per diem allowances. In addition, scientists may present relevant findings at conferences as a means to exchange information with leading authorities in the appropriate fields.

Supplies

The project will require routine laboratory and manufacturing supplies both for ELISA microplate development and lateral flow (rapid) product development.

Vybion - Budget Justification

Vybion is a biotechnology company located in Ithaca, NY with proprietary technologies and 13 years of operational experience in developing biopharmaceutical drugs, vaccines and diagnostic proteins. Vybion has developed over 100 proteins in these areas for a range of private and public companies and maintains both a contract services and proprietary products division. Vybion recently has divested its reagent business under which it had 15 proteins used in research and in diagnostic kits approved by regulatory agencies in the US and Europe. Our contract services division has developed over 40 cytokines, several cancer drugs, four autoimmune based drugs, two vaccines, several other classes of protein drugs and a variety of preclinical and research proteins.

Personnel:
John D. Noti, Ph.D. Director of Research will serve as the project manager and devote EF of his time to the project. He will be the primary contact to coordinate efforts between the various collaborators for Vybion's role on the project. Dr. Noti will oversee efforts at Vybion to clone and express Lassa Virus proteins in different formats and expression systems in order to decrease costs, increase efficiencies and define protein formats needed to detect false negatives. Dr. Noti has over 30 years of experience in transcriptional regulation and gene expression and has been with Vybion for the last three years.

Wallace R. Fish, Ph.D. is a Senior Molecular Biologist with over 30 years of experience. Dr. Fish will lead the laboratory effort in gene/protein expression development and will devote EFF of his time to the project for the first 2 years during which we expect all modifications for expression will be developed. Dr. Fish has been with Vybion for 6 years in developing projects from gene expression strategies through downstream recovery. Dr. Fish spent 10 years in Nairobi, Kenya in East Africa as Project Leader and Senior Scientist for Trypanosome Vaccine Development.

Hui Zhu, MS is a Scientist and Head of Fermentation at Vybion for the past 5 years. Mr. Zhu is responsible for all fermentation development projects and has developed fermentation strategies and optimization for at least 10 drug and at least 20 nondrug. Mr. Zhu has worked with yeast, bacterial and mammalian development. He will be responsible for all optimization development for these systems on the Lassa project. Mr. Zhu joined Vybion from graduate studies at LSU in Baton Rouge having previously worked as a Senior Research Associate at Zhejiang University, Institute of Biotechnology, Hangzhou, China.

Scott Fletcher, BS is a Scientist at Vybion in downstream processing and analytical assay development. Mr. Fletcher has developed downstream process and several analytical assays for Vybion projects over the last 8 years. Mr. Fletcher will be responsible for process and purification of all Lassa proteins devoting EFFORT of his time to the project. He will also be responsible for purification of batches of Lassa proteins in current formats and performing in process QC.

Consultants
Some final QC will be outsourced as part of the supply budget. Predominately this would be MS/MS for protein identification until the process is validated.

Equipment
None requested

Travel
Vybion will fund travel as needed.

Supplies
The current status of the processes for fermentation, processing and recovery of Lassa proteins is expensive and time consuming particularly product like enzymes to cleave fusion proteins. Given the needs for testing, we estimate a minimum of 70-100L of total fermentation volume for all three proteins which can currently only

be done in small batches. Purification costs are currently considerable. The majority of supply costs will be in current production and alternative development in yeast and mammalian cell systems in the first two years after which supply costs are reduced.

Vybion does not anticipate the need for licensing of technologies or other capabilities.

USAMRIID Budget Justification

United States Army Medical Research Institute of Infectious Diseases (USAMRIID), an organization of the U.S. Army Medical Research and Materiel Command, is the lead medical research laboratory for the U.S. Biological Defense Research Program. USAMRIID's military and civilian staff of 750 includes microbiologists, physicians, veterinarians, pathologists, chemists, molecular biologists, virologists, nurses, regulatory scientists, and the technical and administrative staff necessary to support the research. USAMRIID conducts basic and applied research on biological threats resulting in medical solutions to protect military service members. The Institute plays a key role as the only laboratory in the Department of Defense (DoD) equipped to safely study highly hazardous infectious agents requiring maximum containment at biosafety level (BSL)-4.

Personnel

Mary C. Guttieri, Ph.D. will serve as Director of the USAMRIID Subcontract and devote EFF of her time and effort (no direct compensation). She is currently a government service scientist in the Department of Molecular Virology at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) in Frederick, Maryland. She has more than 15 years of experience in virology and, for the past 9 years, has conducted research focused on the production and analysis of virus-specific human monoclonal antibodies of potential therapeutic value. She has extensive experience in developing and implementing research plans for the discovery of immuno-therapeutic bio-defense reagents and is presently funded through the Military Infectious Disease and Biological Defense Research Programs, which utilize a competitive research proposal submission process. Currently, she is the principal investigator for multiple research projects, which focus on the generation of protective measures against viral hemorrhagic fevers and poxvirus infections. Dr. Guttieri's scientific achievements include engineering novel expression systems for antibody development using insect cell expression technology and the implementation of innovative methods for antibody production using phage display and transgenic mouse models. She filed an invention disclosure for an antibody cassette expression system that she engineered for conversion of phage display-selected Fab fragments into complete human IgG1 antibodies in insect cells. Dr. Guttieri maintains multiple extramural collaborations with researchers at academic institutions, non-profit organizations, and in the biotechnology sector. She is a Federal employee and cannot receive direct compensation. She will participate in all aspects of the study and supervise the research of the civilian comtractor.

Civilian Contractor will devote EFFO effort to the proposed study. All live arenavirus culture studies will be performed in the secure BSL-4 laboratories at USAMRIID. This individual will provide irradiated stocks of various arenaviruses necessary to optimize the antigen capture assays and test cross-reactivity and compare the recombinant antigen based Lassa fever diagnostic assays under development with existing BSL-4 Lassa fever diagnostic assays and PCR. This scientist will provide detailed sequence analysis of LASV isolated from clinical samples obtained in West Africa. She will be paid at a rate applicable to Frederick, MD. Fringe Rate is based on TRUE's historical rate of 15%.

Supplies

Cost of supplies as needed for BSL-4 work

Other Expenses

Special immunization program fees to include annual surveillance, VEE primary vaccination/titer/1 booster, RVF/EEE/WEE primary vaccination/titer/1 booster, and smallpox primary, biosafety equipment and BSL-4 consumable supplies. Immunizations are required for the technician to work in the BSL-4 laboratory and there are also personal protection and other equipment needed for work in the BSL-4 laboratory.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Robert F. Garry	Professor, Microbiology and Immunology
eRA COMMONS USER NAME (credential, e.g., agency login)	Assistant Dean for Graduate Studies in Biomedical Sciences

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Indiana State University, Terre Haute, Indiana	B.S.	1974	Life Sciences
University of Texas, Austin, Texas	Ph.D.	1978	Microbiology
University of Texas, Austin, Texas	Postdoctoral		Virology

A. Positions and Honors.

1983 - date Assistant Professor (1983-87); Associate Professor (1987–93); Professor (1993-date) of Microbiology and Immunology at Tulane University School of Medicine at New Orleans, Louisiana 70112.
1995 – date Established (with Dr. David Sander) All the Virology on the World Wide Web (www.virology.net).
2006 – date Assistant Dean for Graduate Studies in Biomedical Sciences
2004 – date Editor-in-Chief: *Virology Journal* (BioMed Central)
1988 - date Member of 6 *Ad hoc* NIH AIDS Study Sections (ARR-3, 1988-90, before charter); Member of NIH AIDS Molecular Biology Study Section (ARR-C, 1990-1996); Member or Chair of 31 NIAID, NHLBI, MBRS, NCI or HHS Special Study sections (1996-date). Chair, VATID Biodefense Study Section (2002); Chair, SBIR-STTR Biodefense Study Section (2002-2006).

B. Selected peer-reviewed publications (in chronological order).

Garry, R.F., Bishop, J.M., Parker, S., Westbrooke, K., Lewis, G., and Waite, M.R.F. (1979). Na+ and K+ and regulation of protein synthesis in Sindbis virus-infected cells. *Virology* **96,** 108-120.

Lewis, R.B., McClure, J., Rup, B.J., Niesel, D., Garry, R.F., Hoelzer [Pierce], J., Nazerian, K., Bose, H.R. (1981). Avian reticuloendotheliosis virus: Identification of the target cell for transformation. *Cell* **25,** 421-432.

Rasheed, S., A. A. Gottlieb, and R.F. Garry (1986). Cell killing by UV-inactivated human immunodeficiency virus. *Virology* **153,** 395-400.

Garry, R.F., Witte, M., Gottlieb, A.A., Elvin-Lewis, M., Gottlieb, M., Witte, C., Alexander, S.S., Cole, W.R., Drake, W.L. (1988). Documentation of an AIDS virus infection in 1968. *JAMA* **260,** 2085-2087.

Gallaher, W.R., Ball, J.M., Garry, R.F., Griffin, M.C., and Montelaro, R.C. (1989). A general model for the TM proteins of HIV and other retroviruses. *AIDS Res. Hum. Retro.* **5,** 431-440.

Qureshi, M.N, Coy, D.H., Garry, R.F., and Henderson, L.A. (1990). Characterization of a putative cellular receptor for the HIV-1 TM glycoprotein using synthetic peptides. *AIDS* **4,** 553-558.

Garry, R.F., and Witte, M. (1990). Early case of AIDS in the United States. *Nature* **347,** 509.

Garry, R.F., Fermin, C.D., Hart, D.J., Alexander, S.S., Donehower, L.A., and Luo-Zhang, H. (1990). Detection of a human intracisternal A-type retroviral particle that is antigenically-related to HIV. *Science* **250,** 1127-1129.

Garry, R.F., and Fermin, C.D. (1993). Viral burden in AIDS. *Nature* **365,** 301-302, 1993.

Voss, T.G., Fermin, C.D., Levy, J.A., Vigh, S., Choi, B., and Garry, R.F. (1996). Alteration of intracellular Na+ and K+ concentrations correlates with induction of cytopathic effects by HIV. *J. Virol.*, **70,** 5447-5454.

Choi, B., Gatti, P.J., Haislip, A.M., and Garry, R.F. (1998). Role of potassium in HIV production and cytopathic effects. *Virology* **247,** 189-199, 1998.

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cells using both apoptotic and necrotic pathways. *AIDS* **13,** 1827-1839.

Plymale, D.R., Comardelle, A., Fermin, C.D., Tencza, S.B., Meitzner, T.A., Montelaro, R.C., and Garry, R.F. (1999). Concentration-dependent differential induction of necrosis or apoptosis by the HIV-1 lytic peptide. *Peptides* **20,** 1275-1283.

Garry, R.F. (2003). Unexpected similarity between the carboxyl termini of lentivirus and pestivirus envelope proteins. *AIDS* **17**, 276-277.

Garry, R. F. and Dash, S. (2003). Proteomics computational analyses suggest that hepatitis C virus E1 and pestivirus E2 envelope glycoproteins are truncated class II fusion proteins. *Virology* **307,** 255-265.

Sainz, B. Jr., Mossel, E.C., Peters, C.J. and Garry, R.F. (2004). Interferon-γ and interferon-γ synergistically inhibit replication of severe acute respiratory syndrome-associated coronavirus. *Virol.* **329,** 11-17.

Garry, C.E., Garry, J.A., and Garry, R.F. (2004). Treatment of warts. *NEJM* ***351,*** 1962.

Jaspan, H.B., Robinson, J.E., Amedee, A.M., Van Dyke, R.B., and Garry, R.F. (2004). Neutralizing antibody to HIV-1 and SIV in amniotic fluid. *J. Clin. Virol.* **31,** 190-197.

Garry C.E., and Garry R.F. (2004) Proteomics computational analyses suggest that the carboxyl terminal glycoproteins of Bunyaviruses are class II viral fusion proteins. *Theor Biol Med Model* **1,** 10.

Sainz, B. Jr., Rausch, J.M. Gallaher, W.R., Garry, R.F., and Wimley, W.C. (2005). The aromatic domain of the coronavirus class I viral fusion protein induces membrane permeabilization: putative role during viral entry. *Biochemistry*, **44,** 947-958.

Sainz, B. Jr., Rausch, J.M. Gallaher, W.R., Garry, R.F., and Wimley, W.C. (2005). Identification and characterization of the putative fusion peptide of the severe acute respiratory syndrome-associated coronavirus (SARS-CoV) spike protein. *J. Virol.,* **79,** 7195-7206.

Hazari S., Patil A., Joshi V., Sullivan D.E., Fermin C.D., Garry R.F., Elliott R.M., and Dash S. (2005). Alpha interferon inhibits translation mediated by the internal ribosome entry site of six different hepatitis C virus genotypes. *J. Gen. Virol.* **86,** 3047-3053.

Fermin C.D. and Garry R.F. (2005). Alterations of lymphocyte membranes during HIV-1 infection via multiple and simultaneous entry strategies. *Microsc Res. Tech.* **68,** 149-67.

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Hrobowski Y.M., Garry R.F., and Michael S.F. (2005). Peptide inhibitors of dengue virus and West Nile virus infectivity. *Virology Journal* **2,** 49.

Colmegna I., Koehler J.W., Garry R.F., and Espinoza L.R. (2006). Musculoskeletal and autoimmune manifestations of HIV, syphilis and tuberculosis. *Curr Opin. Rheumatol.* **18,** 88-95.

Sainz B. Jr., Mossel E.C., Gallaher W.R., Wimley W.C., Peters C.J., Wilson R.B, and Garry R.F. (2006). Inhibition of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) infectivity by peptides analogous to the viral spike protein. *Virus Research* **120,** 146-55.

Fair J.N., Jentes E., Inapogui A. Kourouma K., Goba A., Bah A., Tounkara M., Coulibaly, M., Garry, R. F. and Bausch, D. G. (2007). Lassa Virus-Infected Rodents in Refugee Camps in Guinea: A Looming Threat to Public Health in a Politically Unstable Region, *Vector-borne and Zoonotic Diseases*, **7,** 167-72.

Costin JM, Rausch JM, Garry RF, Wimley WC. (2007). Viroporin potential of the lentivirus lytic peptide (LLP) domains of the HIV-1 gp41 protein. *Virol J.* **4,** 123.

Khan SH, Goba A, Chu M, Roth C, Healing T, Marx A, Fair J, Guttieri MC, Ferro P, Imes T, Monagin C, Garry RF, Bausch DG; for the Mano River Union Lassa Fever Network. (2008). New opportunities for field research on the pathogenesis and treatment of Lassa fever. *Antiviral Res.* **78,** 103-15.

Choi B, Gatti PJ, Fermin CD, Vigh S, Haislip AM, Garry RF. (2008). Down-regulation of cell surface CXCR4 by HIV-1. *Virol J.* **5,** 6.

Colmegna I, Sainz B Jr, Citera G, Maldonado-Cocco JA, Garry RF, Espinoza LR. (2008). Anti-20S Proteasome Antibodies in Psoriatic Arthritis. *J Rheumatol.* **35,** 674-676.

Garry CE, Garry RF. (2008). Proteomics computational analyses suggest that baculovirus GP64 superfamily proteins are class III penetrenes. *Virol J.* **5,** 28

Branco, L., Matschiner, A., Fair, J.N., Goba, A., Ferro, P., Cashman, K., Sampey, D., Schoepp, R., Tesh, R., Bausch, D.B., Garry, RF, and Guttieri, M.C., 2008. Bacterial-based systems for expression and purification of recombinant Lassa virus proteins of immunological relevance. *Virology Journal* 5: 73.

C. Research Support

Ongoing Research Support

1UC1AI067188-01 Garry 10/1/05 – 11/30/08
NIAID
Recombinant antigen multiagent diagnostic assays for Lassa and other arenaviruses. The goals are to develop modern IgM-, IgG- and antigen-capture assays for diagnosis of infection by Old and New World arenaviruses.
Role: PI
The currently proposed project will perform essential steps in the commercial development of prototypic Lassa fever recombinant ELISA assays developed in this previously funded U01, and develop point-of-care Lassa fever recombinant lateral flow assays. No overlap.

1R41AI068221-01 Kolakovsky 12/1/05 – 11/30/08
NIAID
Development of molecular diagnostic assays for systemic autoimmune diseases
The goals are to develop novel assays for SLE and related autoimmune diseases based on the presence of a human A-type retrovirus.
Role: Investigator
No overlap.

1R41AI068230-01 Garry 7/1/06 – 6/30/08
NIAID
Peptide drugs against influenza virus
Synthetic peptides inhibitors of influenza virus, a virus with a class I viral fusion protein, will be developed and tested in vitro and in vivo.
Role: PI
No overlap.

1 R56 AI64617-01A2 Garry 7/1/06 – 6/30/08
NIAID
Peptide inhibitors of dengue virus infectivity
The dengue virus envelope protein is a class II viral fusion protein. Peptides corresponding to sequences in the envelope proteins of dengue virus that inhibit that inhibit fusion/infectivity will be identified and optimized. The efficacy of lead peptides in an appropriate animal challenge models will be evaluated.
Role: PI
No overlap.

Private Garry (PI) 9-1-07 – 7-31-10
Private Source
Design, Delivery and Development of Therapeutic Peptides
Role: PI
No overlap.
This Program Project style grant is a consortium of seven projects and three cores to develop peptide based drugs for treatment of infectious diseases, immunological diseases, cardiovascular disease and cancers. Dr. Garrry is the overall PI, director of one Project on biothreat viruses and the Administrative Core.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Daniel G. Bausch	Associate Professor of Tropical Medicine, School of Public Health and Tropical Medicine, Tulane University
eRA COMMONS USER NAME (credential, e.g. agency login) eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Northwestern University, Evanston, IL	BA	1983	Psychology
Loyola Stritch School of Medicine, Maywood, IL	MD	1989	Medicine
Boston University, Boston, MA	Residency	1992	Internal Medicine
Tulane University, New Orleans, LA	Fellowship	1994	Infectious Disease
Tulane University, New Orleans, LA	MPH&TM	1994	Public Health/Trop Med

A. Positions and Honors

Positions and Employment

1983 – 1985 Laboratory Technician, Department of Clinical Pharmacology, University of Chicago, Chicago, IL

1995 – 1996 Director, Community Medicine and Training Project, International Medical Relief Fund, Suchitoto, El Salvador, Central America

1995 – 1996 Visiting Professor, Departments of Medicine and Microbiology, University of El Salvador, San Salvador, Central America

1996 – 1999 Guest Researcher, Pathogenesis Section, Special Pathogens Branch, CDC, Atlanta, GA

1996 – 1999 Clinical Assistant Professor, Department of Medicine, Section of Infectious Diseases, Tulane Health University Medical Center, New Orleans, LA (based at CDC Atlanta)

1996 – 1999 Research Assistant Professor, Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA (based at CDC Atlanta)

1999 – 2003 Adjunct Professor, Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA (based at CDC Atlanta)

1996 – 2003 Medical Officer (and Acting Chief, 2001-2002), Epidemiology Unit, Special Pathogens Branch, CDC, Atlanta, GA

2003 – present Associate Professor, Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA

Other Experience and Professional Memberships

1992 Board Certified, Internal Medicine

1995 – present Founding Member and Board Member, Doctors for Global Health

1996 Board Certified, Infectious Diseases

1996 – 1999 Faculty Member, Delta Region AIDS Education and Training Center, New Orleans, LA

1996 – 2002 Director, CDC Lassa Fever Research Field Station, Guinea, West Africa

1997 Certificate of Knowledge in Tropical Medicine, American Society of Tropical Medicine and Hygiene

1998 – 1999 Program Committee, American Society of Tropical Medicine and Hygiene

1998 – present Young Investigator Award Committee, American Society of Tropical Medicine and Hygiene

1999 – 2001 Certificate, International Emergency Capacity Development Program, CDC, Atlanta, GA

2001 – 2003 Commander, United States Public Health Service Commissioned Corps

2004 – present Fellow, Center for Evidence-Based Global Health, Tulane School of Public Health and Tropical Medicine, New Orleans, LA

2004 – present Senior Medical-Technical Coordinator, WHO Mano River Union Lassa Fever Network, World Health Organization, Geneva, Switzerland

Honors

1988	Alpha Omega Alpha Medical Honor Society
1994	Dean's Scholarship Award and Valedictorian, Tulane School of Public Health and Tropical Medicine, New Orleans, LA
1994	Delta Omega Public Health Honor Society
1995	Travel Award, American Society of Tropical Medicine and Hygiene
2002	Secretary's Award for Distinguished Service, Uganda Ebola Outbreak Response Team, Department of Health and Human Services
2002, 2003	Foreign Duty Service Awards, United States Public Health Service
2004	Secretary's Award for Distinguished Service, SARS Outbreak Response Team (Vietnam), Department of Health and Human Services

B. Selected Peer-Reviewed Publications (in chronological order)

1. **Bausch DG**, Cline, B. The Impact of Control Measures on Urinary Schistosomiasis in Primary School Children in Northern Cameroon: A Unique Opportunity for Controlled Observations. *Am J Trop Med Hyg* 1995; 53(6):577-580.
2. **Bausch DG**, Rollin P. La Fièvre de Lassa (French). *Annales de l'Institut Pasteur* 1997; 8(3):223-231.
3. **Bausch DG**. Malaria and Other Tropical Infections in the Intensive Care Unit. *In* Shoemaker WC et al. (eds): *Textbook of Critical Care*. Fourth edition, Philadelphia, W.B. Saunders Company, 1999; pp 768-799.
4. Bertherat E, Talarmin A, Zeller H. For the International Scientific and Technical Committee for Marburg Hemorrhagic Fever Control in the Democratic Republic of the Congo. République Démocratique du Congo: Entre Guerre Civile et Virus Marburg [French]. *Med Trop* 2000; 59(2):201-204.
5. **Bausch DG**, Rollin PE, Demby AH, Coulibaly M, Kanu J, Conteh AS, Wagoner KD, McMullan LK, Bowen MD, Peters CJ, Ksiazek TG. Diagnosis and Clinical Virology of Lassa Fever as Evaluated by Enzyme-Linked Immunosorbent Assay, Indirect Fluorescent-Antibody Test, and Virus Isolation. *J Clin Microbiol* 2000; 38(7):2670-2677. http://jcm.asm.org/cgi/content/full/38/7/2670?view=long&pmid=10878062
6. Bowen MD, Rollin PE, Ksiazek TG, Hustad HL, **Bausch DG**, Demby AH, Bajani MD, Peters CJ, Nichol ST. Genetic Diversity Among Lassa Virus Strains. *J Virol* 2000; 74(15):6992-7004. http://jvi.asm.org/cgi/content/full/74/15/6992?view=long&pmid=10888638
7. Mahanty S, **Bausch DG**, Thomas RL, Goba A, Bah A, Peters CJ, Rollin PE. Low Levels of Interleukin-8 and Interferon-Inducible Protein-10 in Serum are Associated with Fatal Infections in Acute Lassa Fever. *J Infect Dis* 2001; 183(12):1713-1721.
8. Demby AH, Inapougui A, Kargbo K, Koninga J, Kourouma K, Kanu J, Coulibaly M, Wagoner KD, Ksiazek TG, Peters CJ, Rollin PE, **Bausch DG**. Lassa Fever in Guinea. II: Distribution and Prevalence of Lassa Virus Infection in Small Mammals. *Vector Borne Zoonotic Dis* 2001; 1(4):283-297.
9. **Bausch DG**, Demby AH, Coulibaly M, Kanu J, Goba A, Bah A, Condé N, Wurtzig HL, Cavallaro KF, Lloyd E, Baldet FB, Cissé SD, Fofonah D, Savané IK, Tolno RT, Mahy B, Wagoner KD, Ksiazek TG, Peters CJ, Rollin PE. Lassa Fever in Guinea. I. Epidemiology of Human Disease and Clinical Observations. *Vector Borne Zoonotic Dis* 2001; 1(4):269-281.
10. **Bausch DG**, Ksiazek TG. Viral Hemorrhagic Fevers Including Hantavirus Pulmonary Syndrome in the Americas. *Clin Lab Med* 2002; 22(4):981-1020, viii.
11. Garrison LE, **Bausch DG**. Hantavirus Pulmonary Syndrome: The Essentials of an Emerging Disease. *MD Consult Infectious Disease,* March 27, 2003 (electronic publication: http://www.mdconsult.com/).
12. Harper S, **Bausch D**. Ebola and Other Viral Hemorrhagic Fevers. In *The NORD Guide to Rare Disorders.* Philadelphia, Lippincott, Williams & Wilkins, 2003.
13. **Bausch DG**, Borchert M, Grein T, Roth C, Swanepoel R, Libande ML, Talarmin A, Bertherat E, Muyembe-Tamfum JJ, Tugume B, Colebunders R, Kondé KM, Pirard P, Olinda LL, Rodier GR, Campbell P, Tomori O, Ksiazek TG, Rollin PE. Risk Factors for Marburg Hemorrhagic Fever in Durba and Watsa, Democratic Republic of the Congo. *Emerg Infect Dis* 2003; 9(12):1531-1537. http://www.cdc.gov/ncidod/eid/vol9no12/03-0355.htm
14. **Bausch DG**, Rollin PE. Responding to Epidemics of Ebola Hemorrhagic Fever: Progress and Lessons Learned from Recent Outbreaks in Uganda, Gabon, and Congo. *In* Scheld WM, Murray BE, and JM Hughes (eds): *Emerging Infections 6*. Washington, D.C., ASM Press, 2004, pp 35-58.

15. Towner JS, Rollin PE, **Bausch DG**, Sanchez A, Crary SM, Vincent M, Lee WF, Spiropoulou CF, Ksiazek TG, Lukwiya M, Kaducu F, Downing R, Nichol ST. Rapid Diagnosis of Ebola Hemorrhagic Fever by Reverse Transcription-PCR in an Outbreak Setting and Assessment of Patient Viral Load as a Predictor of Outcome. *J Virol* 2004; 78(8):4330–4341. http://jvi.asm.org/cgi/content/full/78/8/4330?view=long&pmid=15047846
16. Sanchez A, Lukwiya M, **Bausch D**, Mahanty S, Sanchez AJ, Wagoner KD, Rollin PE. Analysis of Human Peripheral Blood Samples from Fatal and Nonfatal Cases of Ebola (Sudan) Hemorrhagic Fever: Cellular Responses, Virus Load, and Nitric Oxide Levels. *J Virol* 2004; 78(19):10370-10377
17. **Bausch DG**. Malaria and Other Tropical Infections in the Intensive Care Unit. In Shoemaker WC et al. (eds): *Textbook of Critical Care*. Forth edition, Philadelphia, W.B. Saunders Company, 2005, pp 1367-1382
18. Carroll DS, Mills JN, Montgomery JM, **Bausch DG**, Blair PJ, Burans JP, Felices V, Gianella A, Iihoshi N, Nichol ST, Olson JG, Rogers DS, Salazar M, and TG Ksiazek (2005). Hantavirus pulmonary syndrome in central Bolivia: relationships between reservoir hosts, habitats, and viral genotypes. *Am J Trop Med Hyg* 72(1):54-58
19. **Bausch DG**. Marburg and Ebola viruses (2005). PIER: *The Physicians' Information and Education Resource*. American College of Physicians, electronic publication: http://pier.acponline.org/physicians/diseases/d891/d891.html
20. Enria DA, Mills JN, Flick R, Bowen MD, **Bausch D**, Shieh W-J, and CJ Peters. Arenavirus Infections. In Guerrant RL, Walker DH, and PF Weller (eds): *Tropical Infectious Diseases. Principles, Pathogens and Practice*, 2nd Ed. Philadelphia (PA), Elsevier Churchill Livingstone, 2006, pp 734-755
21. Nkoghe D, Formenty P, Leroy ÉM, Nnegue S, Obame Edou SY, Iba Ba J, Allarangar Y, Cabore J, Bachy C, Andraghetti R, de Benoist AC, Galanis E, Rose A, **Bausch D**, Reynolds M, Rollin P, Corera, Shongo R, Gergonne B, Koné LM, Yada A, Roth C and M Toung Mve (2005). Multiple Ebola virus haemorrhagic fever outbreaks in Gabon, from October 2001 to April 2002 [French, English abstract]. *Bull Soc Pathol Exot*, 98 (3): 224-229
22. Reynolds MG, Anh BH, Thu V, Montgomery JM, **Bausch DG**, Shah JJ, Maloney S, Leitmeyer KC, Horby P, Plant AJ, and TM Uyeki (2006). Factors associated with nosocomial SARS-CoV transmission among health care workers in Hanoi, Vietnam, 2003. *BMC Public Health* 6:207. http://www.biomedcentral.com/1471-2458/6/207
23. **Bausch DG**, Nichol ST, Muyembe-Tamfum JJ, Borchert M, Rollin PE, Sleurs H, Campbell P, Tshioko FK, Roth C, Colebunders R, Pirard P, Mardel S, Olinda LA, Zeller H, Tshomba A, Kulidri A, Libande ML, Mulangu S, Formenty P, Grein T, Leirs H, Braack L, Ksiazek T, Zaki S, Bowen MD, Smit SB, Leman PA, Burt FJ, Kemp A, and R Swanepoel for the International Scientific and Technical Committee for Marburg Hemorrhagic Fever Control in the Democratic Republic of the Congo (2006). Marburg hemorrhagic fever associated with multiple genetic lineages of virus. *N Engl J Med.* 355(9):909-919
24. Haynes LM, Miao C, Harcourt JL, Montgomery JM, Le MQ, Dryga SA, Kamrud KI, Rivers B, Babcock GJ, Oliver JB, Comer JA, Reynolds M, Uyeki TM, **Bausch D**, Ksiazek T, Thomas W, Alterson H, Smith J, Ambrosino DM, and LJ Anderson (2007). Recombinant protein-based assays for the detection of antibodies to SARS-CoV spike and nucleocapsid proteins. *Clin Vaccine Immunol.* 14(3):331-3
25. **Bausch DG** and TW Geisbert (2007). Development of vaccines for Marburg hemorrhagic fever. *Expert Rev Vaccines.* 6(1):57-74
26. Fair J, Jentes E, Inapogui A, Kourouma K, Goba A, Bah A, Tounkara M, Coulibaly M, Garry RF, and **DG Bausch** (2007). Lassa virus-infected rodents in refugee camps in Guinea: a looming threat to public health in a politically unstable region. *Vector Borne Zoonotic Dis* 7(2):167-72
27. **Bausch DG**, Towner JS, Dowell SF, Kaducu F, Lukwiya M, Sanchez A, Nichol ST, Ksiazek TG, and PE Rollin (2007). Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. *J Infect Dis* 196(Suppl 2):S142-7
28. **Bausch DG**, Feldmann H, Geisbert TW, Bray M, Sprecher AG, Boumandouki P, Rollin PE, Roth C, and the Winnipeg Filovirus Clinical Working Group (2007). Outbreaks of filovirus hemorrhagic fever: Time to refocus on the patient. *J Infect Dis* 196(Suppl 2):S136-41
29. PE Rollin, **Bausch DG**, and A Sanchez (2007). Blood chemistry measurements and D-dimer levels associated with fatal and nonfatal outcomes in humans infected with Sudan Ebola virus. *J Infect Dis* 196(Suppl 2):S364-71

30. Colebunders R, Tshomba A, Van Kerkhove MD, **Bausch DG**, Campbell P, Libande M, Pirard P, Tshioko F, Mardel S, Mulangu S, Sleurs H, Rollin PE, Muyembe-Tamfum JJ, Jeffs B, and M Borchert on behalf of the International Scientific and Technical Committee DRC Watsa/Durba 1999 Marburg Outbreak Investigation Group (2007). Marburg hemorrhagic fever in Durba and Watsa, Democratic Republic of the Congo: Clinical documentation, features of illness, and treatment. *J Infect Dis* 196(Suppl 2):S148-53
31. Swanepoel R, Smit SB, Rollin PE, Formenty P, Leman PA, Kemp A, Burt FJ, Grobbelaar AA, Croft J, **Bausch DG**, Zeller H, Leirs H, Braack LEO, Libande ML, Zaki S, Nichol ST, Ksiazek TG, and JT Paweska on behalf of the International Scientific and Technical Committee for Marburg Hemorrhagic Fever Control in the Democratic Republic of the Congo (2007). Studies of reservoir hosts for Marburg virus. *Emerg Infect Dis* 12:1847-51. http://www.cdc.gov/eid/content/13/12/1847.htm
32. Khan SH, Goba A, Chu M, Roth C, Healing T, Marx A, Fair J, Guttieri MC, Ferro P, Imes T, Monagin C, Garry RF, and **DG Bausch** for the Mano River Union Lassa Fever Network (2008). New opportunities for field research on the pathogenesis and treatment of Lassa fever. *Antiviral Res* 78 (1):103-115
33. **Bausch DG**, Sprecher AG, Jeffs B, and P Boumandouki (2008). Treatment of Marburg and Ebola hemorrhagic fevers: a strategy for testing new drugs and vaccines under outbreak conditions. *Antiviral Res* 78 (1):150-61

C. Research Support

Ongoing Research Support

1 UC1 AI067188-01 (PI-Garry) 10/01/05 – 09/30/08
NIH/NIAID
Recombinant Antigen Multiagent Diagnostic Assays for Lassa and other Arenaviruses—The goal of this project is to establish new diagnostic assays for the arenaviruses that do depend on reagents requiring production in a P4 laboratory.
Role: Co-Investigator

Private Source (PI-Roth) 08/01/04 – 12/31/09
Private Source
Strengthened Outbreak Alert and Response: Preparedness for Rapid Diagnosis and Control of Lassa Fever in the Mano River Union Region—The goal of this project is to establish a network of laboratories and trained personnel for the rapid diagnosis of Lassa fever and other epidemic prone infectious diseases in Guinea, Sierra Leone and Liberia.
Role: Senior Medical-Technical Coordinator

Private Source (PI-Roth) 01/01/08 – 12/31/09
Establishing Community-Based Surveillance and Diagnosis of Lassa Fever and other Priority Epidemic-Prone Diseases in the Eastern Province of Sierra Leone—The goal of this project is to set-up a surveillance system for Lassa fever and other priority epidemic-prone infectious diseases in the eastern region of Sierra Leone.
Role: Senior Medical-Technical Coordinator

Private (PI-Bausch) 05/01/08 – 04/30/10
Private Source
Private Source –This is intramural funding to explore for the presence of hanta- and other rodent-borne viruses in New Orleans and to enhance overall research capacity for rodent-borne diseases at Tulane.
Role: Principal Investigator

T37 MD001424 (PI-Oberhelman) 07/01/05 – 06/30/08
NIH/National Center on Minority Health and Health Disparities
Minority Health and Health Disparities International Research Training—This training grant provides opportunities for undergraduate minorities to get involved in international research.
Role: Co-Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Luis Branco	Chief Scientific Officer
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Massachusetts, Amherst	B.S.	1995	Microbiology

A. Positions and Honors.

Positions and Employment

1989-1995 Laboratory Supervisor and Research Technician, University of Massachusetts, Amherst, MA
1995-2000 Associate Scientist, MedImmune, Gaithersburg, MD
2000-2004 Senior Research Associate/ Manager, Human Genome Sciences, Rockville, MD
2004-2005 Vice President of Research, BioFactura, Rockville, MD
2005-present Chief Scientific Officer, BioFactura, Rockville, MD

Honors

1992 Becton Dickinson Award for Best Presentation. XIVth Annual Becton Dickinson Symposium. Harvard University, Boston, MA
1997 Director's Award for Excellence, MedImmune, Inc.
1998 Chairman's Award for Excellence, MedImmune, Inc.
1999 Director's Award for Excellence, MedImmune, Inc.
2001 Director's Award for Excellence, MedImmune, Inc.

B. Selected peer-reviewed publications (in chronological order).

1. John P. DeVincenzo, Caroline B. Hall, David W. Kimberlin, Pablo J. Sanchez, William J. Rodriguez, Barbara A. Jantaush, Lawrence Corey, Jeffrey S. Khan, Janet A. Englund, JoAnn A. Suzich, Fran J. Palmer-Hill, Luis Branco, Syd Johnson, Nita K. Patel, Franklin M. Piazza. Surveillance of Clinical Respiratory Syncytial Virus (RSV) Isolates for Palivizumab (Synagis®) Resistant Mutants. J. Infect. Dis. 2004; 190:975-978.

2. Branco L, Barren P, Mao SY, Pfarr D, Kaplan R, Postema C, Langermann S, Koenig S, and Johnson S. Selective Deletion of Antigen-Specific, Activated T Cells by a Humanized mAb to CD2 (MEDI-507) is Mediated by NK Cells. Transplantation 1999; 68 (10): 1588-1596.

3. Branco, L., Matschiner, A., Fair, J.N., Goba, A., Ferro, P., Cashman, K., Sampey, D., Schoepp, R., Tesh, R., Bausch D.B., Garry, RF, and Guttieri, M.C., 2008. Bacterial-based systems for expression and purification of recombinant Lassa virus proteins of immunological relevance. Virology Journal 5: 73.

C. Research Support

Ongoing Research Support

1 UC1 AI067188-01 Garry (PI) 09/30/2005 - 09/29/2008
NIH/ NIAID
Recombinant antigen multiagent diagnostic assays for Lassa and other arenaviruses
Role: Co-Investigator

Department Of Defense SBIR Phase IIContract No.:
W81XWH-06-C-0029 Branco (PI) 10/14/2006 – 10/13/2008
Generation of Stable Eukaryotic Cell Lines Expressing High Yields of Therapeutic Human Antibodies Against Biowarfare Viral Threat Agents

Private Source

Project Title: StableFast Mammalian Expression and Manufacturing System Development
Project Period: Q1 2007 – Q1 2008

Completed Research Support

Private Source 3/19/01 – 12/23/04

Generation and characterization of a panel of novel human monoclonal antibodies that specifically antagonize CCR5 and block HIV-1 entry
The goal of this project was to generate a panel of murine and fully human monoclonal antibodies to the 7-TM chemokine receptor CCR5 that displayed strong blocking properties to HIV-1 entry into human CD4+ T cells. A panel of 38 novel CCR5 mAbs, including 15 fully human mAbs derived from Xenomice were identified and characterized for their ability to specifically bind CCR5, inhibit ligand binding, and inhibit CCR5-dependent HIV-1 infection. One fully human mAb that showed the most potent inhibition of HIV-1 entry into CD4+ T cells was chosen for clinical studies. This mAb inhibits CCR5 dependent entry of HIV-1 viruses representative of clades A-G, inhibits T20 – resistant HIV-1 entry, does not induce ADCC or CDC in vitro, displays a long serum half – life in monkeys, and has high sc bio – availability in mice.
Role: Co-Investigator

Private Source 4/15/96 -3/15/01

Characterization of a humanized mAb to human CD2 (MEDI-507) with unique immunosuppressive properties
The goal of this project was to fully characterize the mechanism of action of MEDI-507 (Siplizumab) in vitro and in vivo, and to identify unmet medical needs that could be satisfied by the use of this immunotherapeutic. MEDI-507 is unique in its ability to selectively delete activated, but not resting T and NK cells expressing CD2 on the surface (Branco L, Barren P, Mao SY, Pfarr D, Kaplan R, Postema C, Langermann S, Koenig S, and Johnson S. Selective Deletion of Antigen-Specific, Activated T Cells by a Humanized mAb to CD2 (MEDI-507) is Mediated by NK Cells. Transplantation 1999; 68 (10): 1588-1596). Thus, MEDI-507 has shown promise as an immunotherapeutic agent in the treatment of conditions such as Psoriasis and acute T-cell lymphomas
Role: Co-Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Fair, Joseph Neal	Science, Technology & Biosafety Project Leader Biological Threat Reduction Program - Ukraine
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Tulane University School of Medicine	PhD	2007	Molecular Virology
Tulane University School of Public Health & Tropical Medicine	MPH	2006	Tropical Medicine
Loyola University	BS	1999	Biology

A. Positions and Honors.

Positions and Employment

2008-Present Project Leader, Southern Research Institute, Drug Discovery Division, Kiev, UKRAINE
2007-2008 Staff Scientist, US Army Medical Research Institute of Infectious Diseases, Virology Division, Frederick, MD, USA
2005-2007 Department of Defense SMART Pre-Doctoral Fellow, US Army Medical Research Institute of Infectious Diseases, Virology Division, Frederick, MD, USA
2003-2007 Tulane University PhD/MPH Pre-Doctoral Fellow in Molecular Virology & Tropical Medicine, Tulane University School of Medicine & US Army Medical Research Institute of Infectious Diseases, New Orleans, LA & Frederick, MD, USA
2001-2003 Laboratory Supervisor, University of Texas Medical Branch, Viral Hemorrhagic Disease Laboratory, Department of Pathology, Galveston, TX, USA
1999-2001 Research Associate/Liaison, Aaron Diamond AIDS Research Center & The Pasteur Institute, Retrovirology Division, Based at the following sites: Covington, LA, USA; Paris, FRANCE; Yaoundé, CAMEROON; Franceville, GABON.

Other Experience and Professional Memberships

2003-Present American Society for Microbiology
2003-Present American Society for Tropical Medicine & Hygiene
2003-Present Scientific Council Member, International Center for Research on Tropical Infections, (French Acronym CIRIT), N'zerekore, Guinea

Honors

2005 Department of Defense Science, Mathematics, & Research for Transformation (SMART) Pre-Doctoral Fellowship
2005 American Society for Microbiology Katrina Grant-in-Aid Award
2006 American Society for Tropical Medical & Hygiene Travel Award

B. Selected peer-reviewed publications (in chronological order).

1. Vanlandingham DL, Schneider BS, Klingler K, **Fair J**, Beasley D, Huang J, Hamilton P, Higgs S. Real-time reverse transcriptase-polymerase chain reaction quantification of West Nile virus transmitted by Culex pipiens quinquefasciatus. Am J Trop Med Hyg. 2004 Jul;71(1):120-3.
2. **Fair J**, Jentes E, Inapogui A, Kourouma K, Goba A, Bah A, Tounkara M, Coulibaly M, Garry RF, Bausch DG. Lassa virus-infected rodents in refugee camps in Guinea: a looming threat to public health in a politically unstable region. Vector Borne Zoonotic Dis. 2007 Summer;7(2):167-71.
3. Khan SH, Goba A, Chu M, Roth C, Healing T, Marx A, **Fair J**, Guttieri MC, Ferro P, Imes T, Monagin C, Garry RF, Bausch DG; Mano River Union Lassa Fever Network. New opportunities for field research on the pathogenesis and treatment of Lassa fever. Antiviral Res. 2008 Apr;78(1):103-15. Epub 2007 Dec 17.
4. Branco LM, Matschiner A, **Fair JN**, Goba A, Sampey DB, Ferro PJ, Cashman KA, Schoepp RJ, Tesh RB, Bausch DG, Garry RF, Guttieri MC. Bacterial-based systems for expression and purification of recombinant Lassa virus proteins of immunological relevance. Virol J. 2008 Jun 6;5(1):74.

C. Research Support

Ongoing Research Support

1UC1AI067188-01 Garry 10/1/05 – 11/30/08
NIAID
Recombinant antigen multiagent diagnostic assays for Lassa and other arenaviruses. The goals are to develop modern IgM-, IgG- and antigen-capture assays for diagnosis of infection by Old and New World arenaviruses.
Role: Investigator
No overlap

GM0092_08_RD PI: Randal J. Schoep 10/1/07 – 9/30/08
US Department of Defense Global Emerging Infections Surveillance and Response System (DoD-GEIS)
"Detection and Identification of Infectious Diseases Requiring High Level Biological Containment"
Role: Co-PI
No overlap
This provides CONUS and OCONUS DoD laboratories with confirmatory diagnostic testing and outbreak support for arthropod-borne and hemorrhagic viral diseases.

Schoepp (PI) 9/01/07-9/31/08
DoD/Global Emerging Infections Systems
Diagnostic Systems Development for High-Containment Pathogens
Role: Co-PI
No overlap

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
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NAME	POSITION TITLE
Wallace R. Fish	Senior Scientist & Director of Technical Services
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
State University of New York, Oswego, NY	B.A.	1969	Biology
State University of New York HSC, Syracuse, NY	Ph.D.	1980	Microbiology /Immunology

A. Positions and Employment

Vybion, Inc. (formerly Viral Therapeutics, Inc.): Ithaca, NY. Preparative scale production of viral and bacterial proteins, viral particles, and biologicals in *Pichia pastoris* and *E. coli*; gene design and cloning, process development. Senior Scientist & Director of Technical Services. 2002 – present.

Veteran's Affairs Medical Center - Syracuse: Syracuse, NY. Dept. of Surgery. Senior Research Associate. 1998 - 2001.

Armauer Hansen Research Institute: Addis Ababa, Ethiopia. Senior Scientist. 1997.

State University of New York - Health Science Center at Syracuse: Dept. of Biochemistry & Molecular Biology. Research Assistant Professor. 1993 - 1994. Dept. of Pediatrics, Research Assistant Professor. 1994 - 1997.

International Laboratory for Research on Animal Diseases: Nairobi, Kenya, East Africa. Scientist and Project Leader of "Trypanosome Vaccine Development". 1983 - 1993.

Uniformed Services University of the Health Sciences: Dept. of Preventive Medicine and Biometrics, Bethesda, MD; Senior Research Associate. 1982 - 1983.

St. Louis University: Depts. of Medicine and Microbiology, School of Medicine, St. Louis, MO; postdoctoral fellow. 1980 - 1982.

The Rockefeller University: Laboratory of Parasitology, New York, NY; postdoctoral fellow. 1979 - 1980.

State University of New York - Health Science Center at Syracuse: Dept. of Microbiology and Immunology, Syracuse, NY; research assistant, teaching assistant and graduate student. 1974 - 1979.

Indiana University of Pennsylvania: Indiana, PA. Biology Dept.; laboratory instructor and graduate student. 1969 - 1970.

B. Publications

1) W.R. Fish, D.H. Beach and G.G. Holz, Jr. (1978). Cultivation of trypanosomatids. J. Parasitol., **64**: 546-547.
2) W.R. Fish, G.G. Holz, Jr., D.H. Beach, E. Owen and G.E. Anekwe (1981). The cyclopropane fatty acid of Trypanosomatids. Molec. Biochem. Parasitol., **3**: 103-115.
3) W.R. Fish, G.G. Holz, Jr., and D.H. Beach (1982). Some *Phytomonas* and *Herpetomonas* species form unique *iso*-branched polyunsaturated fatty acids. Molec. Biochem. Parasitol., **4**: 1-18.
4) W.R. Fish, J.J. Marr, and R.L. Berens (1982). The purine metabolism of *Trypanosoma gambiense*. Biochim. Biophys. Acta, **714**: 422-428.

5) W.R. Fish, D.L. Looker, J.J. Marr and R.L. Berens (1982). Purine metabolism in the bloodstream forms of *Trypanosoma gambiense* and *Trypanosoma rhodesiense*. Biochim. Biophys. Acta, **719**: 223-231.
6) K.-P. Chang and W.R. Fish (1983). *Leishmania*. IN: "*In Vitro* Cultivation of Protozoan Parasites of Man and Domestic Animals", J.B. Jensen, Ed., CRC Press, 111-153.
7) G.G. Holz, Jr., D.H. Beach, B.N. Singh and W.R. Fish (1983). Biosynthesis of the novel fatty acid, 17-methyl-*cis*-9,10-methyleneoctadecanoic acid, by the parasitic protozoan *Herpetomonas megaseliae*. Lipids, **18**: 607-610. **8)** L.W. Scheibel, E. Bueding, W.R. Fish and J.T. Hawkins (1984). Protease inhibitors and antimalarial effects. Prog. Clin. Biol. Res. **155**: 131-142.
9) W.R. Fish, J.J. Marr, R.L. Berens, D.L. Looker, D.J. Nelson, S.W. LaFon and A.E. Balber (1985). Inosine analogs as chemotherapeutic agents for African trypanosomes: metabolism in trypanosomes and efficacy in tissue culture. Antimicro. Agents Chemother., **27**: 33-36.
10) W.R. Fish, R.T. Nelson, and H. Hirumi (1987). Cell adhesion in *Trypanosoma*: *In vitro* studies of the interaction of *Trypanosoma vivax* with immobilized organic dyes. J. Protozool., **34**: 457-464.
11) D.J. Grab, P. Webster, S. Ito, W.R. Fish, Y. Verjee and J.D. Lonsdale-Eccles (1987). Subcellular localization of a variable surface glycoprotein phosphatidyinositol-specific phospholipase C in African trypanosomes. J. Cell Biol., **106**: 737-746.
12) W.R. Fish, C.W. Muriuki, A.M. Muthiani, D.J. Grab and J.D. Lonsdale-Eccles (1989). Disulfide bond involvement in the maintenance of the cryptic nature of the cross-reacting determinant of metacyclic forms of *Trypanosoma congolense*. Biochemistry, **28**: 5415-5421.
13) W.R. Fish, D.J. Grab and J.D. Lonsdale-Eccles (1989). The cross-reacting determinant of the variable surface glycoprotein of metacyclic *Trypanosoma congolense*. IN: "Protein Traffic in Parasites and Mammalian Cells", J.D. Lonsdale-Eccles, Ed., ILRAD, 42-44.
14) P. Webster and W.R. Fish (1989). Endocytosis in African trypanosomes. II. Occurrence in different life cycle stages and intracellular sorting. Eur. J. Cell Biol., **49**: 303-310.
15) Z.R. Mbawa, I.D. Gumm, W.R. Fish and J.D. Lonsdale-Eccles (1991). Endopeptidase variations among different life-cycle stages of African trypanosomes. Eur. J. Biochem., **195**: 183-190.
16) E.J. Bienen, P. Webster and W.R. Fish (1991). *Trypanosoma (Nannomonas) congolense*: changes in respiratory metabolism during the life cycle. Exp. Parasitol., **73**: 403-412.
17) Y. Eshita, T. Urakawa, H. Hirumi, W.R. Fish and P.A.O. Majiwa (1992). Metacyclic form-specific variable surface glycoprotein genes of *Trypanosoma (Nannomonas) congolense*. Gene, **113**: 139-148.
18) L.K. Read, A.N.K. Jacob, W.R. Fish, A.M. Muthiani and K. Stuart (1993). Sequences of three *Trypanosoma congolense* maxicircle genes allow prediction of regions encoding transcripts that undergo extensive RNA editing. Mol. Biochem. Parasitol., **60**: 337-342.
19) L.K. Read, W.R. Fish, A.M. Muthiani and K. Stuart (1993). Maxicircle DNA and edited mRNA sequences of closely related trypanosome species: implications of kRNA editing for evolution of maxicircle genomes. Nucleic Acids Res., **21**: 4073-4078.
20) D.J. Grab, M.K. Shaw, C.W. Wells, Y. Verjee, D.C.W. Russo, P. Webster, J. Naessens and W.R. Fish (1993). The transferrin receptor in African trypanosomes: identification, partial characterization and subcellular localization. Eur. J. Cell Biol., **62**: 114-126.
21) J.A. Ellis, W.R. Fish, M. Sileghem and F. McOdimba (1993). A colorimetric assay for trypanosome viability and metabolic function. Vet. Parasitol., **50**: 143-149.
22) L.K. Read, K.A. Stankey, W.R. Fish, A.M. Muthiani and K. Stuart (1994). Developmental regulation of RNA editing and polyadenylation in four life cycle stages of *Trypanosoma congolense*. Mol. Biochem. Parasitol., **68**: 297-306.
23) H. Parker, T. Hill, K. Alexander, N. B. Murphy, W. R. Fish and M. Parsons (1995). Three genes and two isozymes: gene conversion and the compartmentalization and expression of the phosphoglycerate kinases of *Trypanosoma (Nannomonas) congolense*. Mol. Biochem. Parasitol., **69**: 269-279.
24) W.R. Fish, Z.M. Nkhungulu, C.W. Muriuki, D.M. Ndegwa, J. D. Lonsdale-Eccles and J. Steyaert (1995). Primary structure and partial characterization of a life-cycle regulated cysteine protease from *Trypanosoma (Nannomonas) congolense*. Gene, **161**: 125-128.
25) W.R. Fish (1995). *Lipid and Membrane Metabolism of the Malaria Parasite and the African Trypanosome*. IN: Biochemistry and Molecular Biology of Parasites, J.J. Marr and M. Muller, Eds. Academic Press, London, 133-45.
26) M. Sobel, W.R. Fish, N. Toma, S. Luo, K. Bird, K. Mori, S. Kusumoto, S.D. Blystone and Y. Suda (2001). Heparin modulates integrin function in human platelets. J. Vasc. Surg., **33**, 587-94.
27) M.S. Da Silva, J.A. Horton, J.M. Wijelath, L.W. Blystone, W.R. Fish, E. Wijelath, K. Strand, S.D. Blystone, and M. Sobel (2003). Heparin modulates integrin-mediated cellular adhesion: Specificity of interactions with β and β integrin subunits. Cell Commun. Adhes., **10**, 59-67.
28) A.C. Fisher, J.-Y. Kim, D. Tullman-Ercek, W. Fish, L.A. Henderson and M.P. DeLisa (2007). Exploration of twin-arginine translocation for expression and purification of correctly folded proteins in *Escherichia coli*. Microbial Biotechnology, Submitted.

C. Research Support

As a scientist working in industry all research and product development has been company funded.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Mary C. Guttieri	Microbiologist, USAMRIID
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Molloy College, Rockville Centre, NY	B.S.	1985	Biology
University of Massachusetts, Amherst, MA	Ph.D.	1995	Microbiology
Postdoctoral Training, USAMRIID, Fort Detrick, MD		1995-1998	

A. Positions and Honors,

2007-present Member of the External Advisory Board for the Louisiana Peptide Translational Research Group

2005-Present Department of Defense International Science and Technology Center Collaborator, as per the Cooperative Threat Reduction Directorate of the Defense Threat Reduction Agency and the U.S. Department of Health and Human Services Biotechnology Engagement Program

2005-Present Committee member of the National Interagency Biodefense Campus Scientific Forum

2001-Present Advisor in the National Research Council Research Associateship Program through the National Academy of Sciences, qualified to serve as an advisor for National Research Council postdoctoral fellows

2001 Co-chairman, Scientific Session F; Hanta and Hepatitis Viruses, American Society of Tropical Medicine and Hygiene 50th Annual Meeting, Atlanta, GA

1999-Present Biodefense Research Microbiologist: Staff Scientist
Department of Molecular Virology, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD

1999-Present Member of the Scientific Steering Committee for the Military Infectious Disease Research Program Science and Technology Evaluation Plan T, Research on Hemorrhagic Fever and Other Highly Lethal Viruses

1998-1999 Scientific government contractors: Scientist II
Sherikon, Inc., Frederick, MD and the Department of Molecular Virology, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD

1995-1998 National Research Council Research Fellowship

B. Selected peer-reviewed publications (in chronological order).

Guttieri, M.C. and Burand, J.P., 1996. Nucleotide sequence, temporal expression, and transcriptional mapping of the p34 late gene of the Hz-1 insect virus. *Virology* 223: 370-75.

Liang, M., Guttieri, M., Lundkvist, A., and Schmaljohn, C., 1997. Baculovirus expression of a human G2-specific, neutralizing IgG monoclonal antibody to Puumala virus. *Virology* 235: 252-60.

Guttieri, M.C. and Schmaljohn, C.S., 2000. Bunyaviridae. In *Embryonic Encyclopedia of Life Sciences*, Nature Publishing Group, London: www.els.net.

Guttieri, M.C., Bookwalter, C., and Schmaljohn, C., 2000. Expression of a human, neutralizing monoclonal antibody specific to Puumala virus G2-protein in stably-transformed insect cells. *J. Immunol. Methods* 246: 97-108.

Guttieri, M.C. and Burand, J.P., 2001. Location, nucleotide sequence, and regulation of the p51 late gene of the Hz1 insect virus: identification of a putative late regulatory element. *Virus Genes* 23: 17-25.

Guttieri, M.C., Sinha, T., Bookwalter, C., Liang, M., and Schmaljohn, C., 2003. Cassette vectors for conversion of Fab fragments into full-length human IgG1 monoclonal antibodies by expression in stably transformed insect cells. *Hybridoma and Hybridomics* 22: 135-46.

Guttieri, M.C. and Liang, M., 2003. Human antibody production using insect cell expression systems. In *Methods in Molecular Biology, Vol. 248, Antibody Engineering: Methods and Protocols* (B.K.C. Lo, ed), The Humana Press, New Jersey, pp. 269-99.

Geisbert, T.W., Jones, S., Fritz, E.A., Shurtleff, A.C., Geisbert, J.B., Liebscher, R., Grolla, A., Stroher, U., Fernando, L., Daddario, K.M., Guttieri, M.C., Mothe, B.R., Larsen, T., Hensley, L.E., Jahrling, P.B., and Feldmann, H., 2005. Development of a new accelerated vaccine for the prevention of Lassa fever. *PLoS Medicine* 2: 537-45.

Spik, K., Shurtleff, A., McElroy, A., Guttieri, M.C., Hooper, J.W., and Schmaljohn, C., 2006. Immunogenicity of combination DNA vaccines for Rift Valley fever virus, tick-borne encephalitis virus, Hantaan virus, and Crimean Congo hemorrhagic fever virus. *Vaccine* 24:4657-66.

Bolken, T.C., Laquerre, S., Zhang, Y., Bailey, T.R., Pevear, D.C., Collett, M.S., Kickner, S.S., Sperzel, L.E., Jones, K.F., Warren, T.K., Lund, S.A., Kirkwood-Watts, D.L., King, D.S., Shurtleff, A.C., Guttieri, M.C., Deng, Y.,Bleam, M., and Hruby, D.E., 2006. Identification and characterization of potent small molecule inhibitor of hemorrhagic fever New World arenaviruses. *Antiviral Research* 69: 86-97.

Ramanathan, H.N., Chung, D.H., Plane, S.J., Sztul, E., Chu, Y.K., Guttieri, M.C., McDowell, M., Ali, G., and Jonsoon, C.B., 2007. Dynein-dependent transport of the Hantaan virus nucleocapsid protein to ERGIC. Journal of *Virology* 81: 8634-8647.

Khan, S.H., Goba, A., Chu, M., Roth, C., Healing, T., Marx, A., Fair, J., Guttieri, M.C., Ferro, P., Imes, T., Monagin, C., Garry, R.F., and Bausch, D., 2008. New opportunities for research in the pathogenesis and treatment of Lassa Fever. *Antiviral Research* 78: 103-115.

Branco, L., Matschiner, A., Fair, J.N., Goba, A., Ferro, P., Cashman, K., Sampey, D., Schoepp, R., Tesh, R., Garry, RF, and Guttieri, M.C., 2008. Mammalian- and bacterial-based systems for expression and purification of recombinant Lassa virus proteins of immunological relevance. *Virology Journal* 5: 73.

C. Research Support

Ongoing Research Support

1UC1AI067188-01 Garry 10/1/05 – 11/30/08
NIAID
Recombinant antigen multiagent diagnostic assays for Lassa and other arenaviruses. The goals are to develop modern IgM-, IgG- and antigen-capture assays for diagnosis of infection by Old and New World arenaviruses.
Role: Investigator
No overlap

BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
F. Jon Geske	Project Director, R&D Corgenix Medical Corporation
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of California, Davis	B.S.	1988	Genetics
University of Colorado Health Sciences Center, Denver	Ph.D.	2000	Experimental Pathology

A. Positions and Employment

- 1988-1990: Laboratory Technician, Department of Medical Pathology, University of California, Davis
- 1990-1992: Laboratory Technician II, Department of Botany, University of California, Davis
- 1992-1994: Research Assistant II, AMC Cancer Research Center, Denver, CO
- 1994-2000: Graduate student, Department of Pathology, University of Colorado Health Sciences Center, Denver
- 2000-2003: Postdoctoral Research Fellow, Department of Pediatrics, National Jewish Medical and Research Center, Denver
- 2003-2005: Laboratory Director, ELISATech, Aurora, CO
- 2005-2006: Research Scientist, Corgenix Medical Corporation, Broomfield, CO
- 2006-present: Project Director, Corgenix Medical Corporation, Broomfield, CO

B. Publications

- **Geske, F.J.**, Guyer, K.E., and Ens, G. (2008). AspirinWorks®: A new immunologic diagnostic test for monitoring aspirin effect. Molecular Diagnosis and Therapy 12 (1): 51-54.
- Expert commentary in "The year in IVDs" *Immunoassay* section (2007). IVD Technology 13 (8): 18.
- **Geske, F.J.**, Muncy, I.J., Tew, D.J., and Lopez, L.R. (2007). An ELISA for determining aspirin effect from urine. IVD Technology 13 (6): 55-64.
- Monks, J., Rosner, D., **Geske, F.J.**, Lehman, L., Hanson, L., Neville, M.C., and Fadok, V.A. (2005). Epithelial cells as phagocytes: apoptotic epithelial cells are engulfed by mammary alveolar epithelial cells and repress inflammatory mediator release. Cell Death and Differentiation 12: 107-114.
- Monks, J., **Geske, F.J.**, Lehman, L., and Fadok, V.A. (2002). Do inflammatory cells participate in mammary gland involution? Journal of Mammary Gland Biology and Neoplasia 7: 163-176.
- **Geske, F.J.**, Monks, J., Lehman, L., and Fadok, V.A. (2002). The role of the macrophage in apoptosis: hunter, gatherer, and regulator. International Journal of Hematology 76: 16-26.
- **Geske, F.J.** and Gerschenson, L.E. (2001). The biology of apoptosis. Human Pathology 32: 1029-1038.
- Gerschenson, L.E. and **Geske, F.J.** (2001). Virchow and apoptosis. American Journal of Pathology 158 : 1543.
- **Geske, F.J.**, Lieberman, R., Strange, R., and Gerschenson, L.E. (2001). Early stages of p53-induced apoptosis are reversible. Cell Death and Differentiation 8: 182-191.
- **Geske, F.J.**, Nelson, A.C., Lieberman, R., Strange, R., Sun, T., and Gerschenson, L.E. (2000). DNA repair is activated in early stages of p53-induced apoptosis. Cell Death and Differentiation 7: 393-401.

- Strange, R., Friis, R.R., Bemis, L.T. and **Geske, F.J.** (1995). Programmed cell death during mammary gland involution. In *Methods in Cell Biology: Cell Death,* vol. 46, eds. Schwartz, L.A. and Osborne, B.A. (Academic Press, Orlando): 355-368.
- Bemis, L.T., **Geske, F.J.** and Strange, R. (1995). Use of the yeast two-hybrid system for identifying the cascade of protein interactions resulting in apoptotic cell death. In *Methods in Cell Biology: Cell Death*, vol. 46, eds. Schwartz, L.A. and Osborne, B.A. (Academic Press, Orlando): 139-151.
- Li, F., Bielke, W., Ke, G., Andres, A-C., Jaggi, R., Saurer, S., Friis, R.R., Niemann, H., Bemis, L.T., **Geske, F.J.** and Strange, R. (1995). Isolation of cell death-associated cDNAs from involuting mouse mammary epithelium. Cell Death and Differentiation 2: 113-122.
- Dickson, R., Larsen, B., Viitanen, P.V., Tormey, M.B., **Geske, J.**, Strange, R. and Bemis, L.T. (1994). Cloning, expression, and purification of a functional nonacetylated mammalian mitochondrial chaperonin 10. Journal of Biological Chemistry 269: 26858-26864.
- Theg, S.M. and **Geske, F.J.** (1992). Biophysical characterization of a transit peptide directing chloroplast protein import. Biochemistry 31: 5053-5060.

C. Research Support

As a scientist working in industry all research and product development has been company funded. Product development conducted or managed has included:

- Development of the AspirinWorks® Test Kit (for determination of aspirin effectiveness)
- Initial development of Lassa antigen-, IgG-, and IgM-capture ELISAs (NIH Prime Award #1UC1AIO67188-01)

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
John D. Noti, Ph.D.	Director, R&D Vybion, Inc.
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Utica College of Syracuse University, Utica, NY	B.S.	1972	Biology
Purdue University, W. Layfayette, Indiana	Ph.D.	1979	Molecular Genetics
Botce Thompson Institute for Plant Biology, Cornell University, Ithica, NY	Postdoctoral	1979-83	Plant Molecular Biology

A. Positions and Employment

1984-1987: Senior Research Scientist at Boyce Thompson Institute. Cloning genes involved in nitrogen fixation.

1987-1988: Research Scientist at Triton Biosciences Inc., Alameda, CA. Cloning HTLV-I provirus from human cells. Expression of HTLV-I genes in E. coli, yeast and human cells to develop a diagnostic assay.

1988-1989: Research Associate at Guthrie Research Institute, Laboratory of Tumor Immunology
Cloning the human ζ and ζ genomic sequences of the Leukocyte Integrin adhesion molecules.

1989-1990: Assistant Scientist at Guthrie Research Institute, continuation of previous year.

1990-1997: Assistant Scientist, Director, Laboratory of Molecular Biology, Guthrie Research Institute, Sayre, PA . Adjunct Scientist at SUNY Health Science Ctr., Syracuse, NY. Transcriptional regulatory studies of the Leukocyte Integrins. Studies of integrins and novel genes associated with breast tumor metastasis.

1997-2004: Associate Scientist, Director, Laboratory of Molecular Biology, Guthrie Research Institute, Adjunct Scientist at SUNY Health Science Ctr., Syracuse, NY. Continuation of previous year.

2004-2005: Associate Scientific Director and Senior Scientist, Guthrie Research Institute, Sayre, PA, Adjunct Scientist at SUNY Health Science Ctr., Syracuse, NY. Continuation of previous year.

2005-present: Director of Research and Development, Vybion Inc., Ithaca, NY.

B. Publications (partial listing of 45 total)

Legocki, R.P., J.D. Noti, M.N. Jagadish, A.C. Yun and A.A. Szalay. (1985). Genetic manipulation of Rhizobium-plant cell communication: Prospects for delivery of foreign gene products to plants. In: Chemistry of Natural Products. ed. Zalewski (Elsevier Publ. B.V., Amsterdam, The Netherlands). pp. 629-642.

Noti, J.D., B. Dudas and A.A. Szalay. (1985). Isolation and characterization of nodulation genes from Bradyrhizobium sp. (Vigna) strain IRc 78. Proc. Natl. Acad. Sci. 82, 7379-7383.

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Kestler, D., L.A. Henderson., and J. Noti. (1993). Construction and expression of recombinant HIV-1 gp41 constructs in procaryotes. Biotechnology and Bioengineering 40, 81-86.

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Strassheim, D., L.G. May, K.A. Varker, H.L. Puhl, S.H. Phelps, R.A. Porter, R.S. Aronstam, J.D. Noti, and C.L. Williams. (1999) M3 muscarinic acetylcholine receptors regulate cytoplasmic myosin by a process involving rhoA and requiring conventional protein kinase C isoforms. J. Biol. Chem., 274(26), 18675-18685.

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Noti, J. D., A. K. Johnson, J. Shea, L. A. Henderson (submitted) Myeloid-specific induction of the leukocyte integrin gene CD11c by Gut-enriched Kruppel-like factor 4.

C. Research Support

NIDA Phase II Small Business Inovation Research (SBRI) Norman, A. (P. I.), Henderson, L. A. and Noti, J. D. (Co-PIs) 1/1/06-12/21/07)

NIH CFDA 93.279
A Human Antibody as an Immunotherapy for Cocaine Abuse
This project is to clone and stably express a novel cocaine antibody gene in CHO cells for large-scale production and eventual clinical evaluation.

1 R01HL63891-04 Noti, J. D. (P.I.) 8/18/01-8/15/07
NIH/NHLBI
Molecular Mechanisms That Regulate CD11d Expression
This project examines the transcriptional mechanisms that regulate the myeloid-specific leukocyte integrin gene CD11d.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Svanas, Gregory	Project Director, Corgenix, Inc.
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Austin College (Sherman, Texas)	BA	1978	Biology, Chemistry
Purdue University (W. Lafayette, Indiana)	Ph.D.	1984	Biochemistry

Please refer to the application instructions in order to complete sections A, B, and C of the Biographical Sketch.

A. Positions and Honors

1984 – 1987	Postdoctoral training	University of Pittsburgh, Pittsburgh, PA
1987 – 1992	Research Scientist	Bayer USA, Elkhart, IN
1992 – 1993	Scientist	Carter-Wallace, Inc., Cranbury, NJ
1993 – 1996	Director of Operations	Spectral Diagnostics, Toronto, Ontario Canada
1996 – 1997	Director of Assay Development	DDx, Inc., Denver, CO
1997 – 2006	Associate Director, R&D	Inverness Medical Innovations, Louisville, CO
2006 – present	Project Director	Corgenix, Inc., Broomfield, CO

B. Selected peer-reviewed publications

1. Teel LD, Daly JA, Jerris RC, Maul D, Svanas G, O'Brien AD, Park CH. "Rapid detection of Shiga toxin-producing Escherichia coli by Optical Immunoassay." J Clin Microbiol. 2007 Aug 45:3377-3380.

2. Greco TP, Conti-Kelly AM, Matsuura E, Greco, Jr. T, Dier KJ, Svanas G, Doyle R, Lopez L. "Antiphospholipid Antibodies in Patients with Coronary Artery Disease." Ann NY Acad Sci 2007 Jun; 1108:466-74.

3. Penttila K, Penttila I, Bonnell R, Kerth P, Koukkunen H, Rantanen T, Svanas G. "Comparison of the troponin T and troponin I ELISA tests, as measured by microplate immunoassay techniques, in diagnosing acute myocardial infarction." Eur J Clin Chem Clin Biochem. 1997 Oct; 35(10): 767-74.

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5. Adler M, Gavaler JS, Duquesnoy R, Fung JJ, Svanas G, Starzl TE, van Thiel DH. "Relationship between the diagnosis, preoperative evaluation, and prognosis after orthotopic liver transplantation." Ann Surg. 1988 Aug; 208(2): 196-202.

6. Kahn D, Svanas GW, Eagon PK, Makowka L, Podesta L, Chapchap P, Starzl TE, Van Thiel DH. "Effect of an antiandrogenic H2 receptor antagonist on hepatic regeneration in rats." J Lab Clin Med. 1988 Aug; 112(2): 232-9.

7. Kahn D, Svanas GW, Eagon PK, Elm M, Porter LE, Makowka L, Podesta L, Chapchap P, Starzl TE, Van Thiel DH. "Liver regeneration in rats treated with the antiandrogen flutamide." J Invest Surg. 1988; 1(2): 133-8.

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9. Kam I, Lynch S, Svanas G, Todo S, Polimeno L, Francavilla A, Penkrot RJ, Takaya S, Ericzon BG, Starzl TE, et al. "Evidence that host size determines liver size: studies in dogs receiving orthotopic liver transplants." Hepatology 1987 Mar-Apr; 7(2): 362-6.

10. Svanas GW, Weiner H. "Enzymatic requirement for cyanamide inactivation of rat liver aldehyde dehydrogenase." Biochem Pharmacol. 1985 Apr 15; 34(8): 1197-204.

11. Svanas GW, Weiner H. "Use of cyanamide to determine localization of acetaldehyde metabolism in rat liver." Alcohol. 1985 Jan-Feb; 2(1): 111-5.

12. Svanas GW, Weiner H. "Aldehyde dehydrogenase activity as the rate-limiting factor for acetaldehyde metabolism in rat liver." Arch Biochem Biophys. 1985 Jan; 236(1): 36-46.

13. Svanas GW, Weiner H. "Identification of aldehyde dehydrogenase resistant to cyanamide and disulfiram inhibition." Alcohol 1984 Jul-Aug; 1(4): 347-9.

14. Svanas GW, Weiner H. "Rapid purification of dehydrogenases by affinity chromatography with ternary complexes." Anal Biochem. 1982 Aug; 124(2): 314-9.

C. Research Support

NIAID 1 UC1 AIO67188-01 "Recombinant Antigen Multivalent Diagnostic Assays for Lassa and Other Arenaviruses"

As a scientist working in industry the remainder of my research and product development has been company-funded.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Wilson, Russell B.	President and Chief Science Officer
eRA COMMONS USER NAME eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Baylor University	B.S.	1981	Biology
University of Texas at Dallas	M.S.	1984	Biology
University of Texas at Dallas	Ph.D.	1988	Molec. Cell Biol.
Carnegie Mellon University	Post-Doc	1987-1991	Cell Biology

A. Positions and Honors.

Positions and Employment

1981 Research Assistant, Department of Biology, Baylor University
1982-1987 Graduate Research Assistant, Department of Biology, Univ. Texas at Dallas
1987-1990 Postdoctoral Research Associate, Center for Fluorescence Research in Biomedical Sciences, Department of Biological Sciences, Carnegie Mellon University
1990-1991 Research Biologist, Center for Fluorescence Research in Biomedical Sciences, Department of Biological Sciences, Carnegie Mellon University
1991-1995 Assistant Professor, Department of Pathology and Laboratory Medicine, Tulane University School of Medicine
1995-present President, Autoimmune Technologies, LLC
2002-present Chief Science Officer, Autoimmune Technologies, LLC
2003-present Member, Southeast Louisiana Veterans Health Care System, Research & Development (COSF) Subcommittee for Research Biosafety

Honors

Robert A. Welch Fellowship, University of Texas at Dallas.
Travel award to attend the International Conference on Analytical Cytology XIII, Society for Analytical Cytology, 1988.
Lawton Chiles Biotechnology Postdoctoral Fellowship, National Institutes of Health.
American Heart Association Fellowship, Pennsylvania Affiliate

B. Selected peer-reviewed publications (in chronological order).

1. Mozola, M., Wilson, R. B., Jordan, E. M., Draper, R. K., and Clowes, R.C. (1984). Cloning and Expression of a Gene Segment Encoding the Enzymatic Moiety of Pseudomonas aeruginosa Exotoxin A. J. Bacteriol. 159:68-687.
2. Clowes, R.C., Mozola, M.A., Wilson, R.B., Hwang, S.R. and Draper, R.K. (1985). Cloning of an Enzymatically Active Segment of the Exotoxin a Gene of Pseudomonas aeruginosa. In Plasmids in Bacteria (Helinski, D.R., Cohen, S.N., Clewel, D.B., Hollander, D.A., eds.). Plenum Publishing Company. pp. 777-790.
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4. Wilson, R.B. and Murphy, R.F. (1989). Flow Cytometric Analysis of Endocytic Compartments. Meth. Cell Biol. 31:293-317.

5. Chaudry, G.J., Wilson, R.B., Draper, R.K. and Clowes, R.C. (1989). A Dipeptide Insertion in Domain I of Exotoxin A that Impairs Receptor Binding. J. Biol. Chem. 264-15151-15156.
6. Roederer, M.J., Barry, J.R., Wilson, R.B. and Murphy, R.F. (1990). Reconstitution of Endosome Lysosome Maturation In Vitro. Eur.J.Cell.Biol. 51:229-234.
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8. Murphy, R.F., Roederer, M., Sipe, D.M., Cain, C.C. and Wilson, R.B. (1992). Endosomal pH Regulation and the Maturation Model for Lysosome Biogenesis. In: Endocytosis: From Cell Biology to Health, Disease and Therapy Courtoy, P.J. (ed.), SpringerVerlag, pp. 91-95.
9. Wiser, M.F., Faur, L.V., Lanners, N., Kelly, M. and Wilson, R.B. (1993). Accessibility and Distribution of Intraerythrocytic Antigens of Plasmodium- Infected Erythrocytes Following Mild Glutaraldehyde Fixation and Detergent Extraction. Parasit. Res. 79:579-586.
10. Wilson, R.B., Mastick, C.C., and Murphy, R.F. (1993). A Chinese Hamster Ovary Cell Line with a Temperature Conditional Defect in Receptor Recycling is Pleiotropically Defective in Lysosome Biogenesis. J. Biol. Chem. 268:25357-25363.
11. Bucci, M., Brown, C.M., Moyer, T.W., Wilson, R.B., and Murphy, R.F. (1994) The Receptor Recycling and Lysosome Biogenesis Mutant TfTI.II Belongs to a New Complementation Group, END 6. Somat. Cell Molec. Genet. 20:47-54.
12. Karukonda, S.R.K., Thompson, H.W., Beuerman, R.W., Lam, D.S.C., Wilson, R., Chew, S.J., and Steinemann, T.L. (1995). Cell Cycle Kinetics in Ptelygium at three latitudes. Brit. J. Ophthalmol. 79:313-317.
13. Rondon, I.J., Scandurro, A.B., Wilson, R.B., and Beckman, B.S. (1995). Changes in Redox Affect the Activity of Elythropoietin RNA Binding Protein. FEBS Letters 359:267-270.
14. Malia, C.M., Jeter, Jr., J.R., Fields, A.P., Wilson, R.B., and Beckman, B.S. (1995). Protein Kinase C αfrom Friend Erythroleukemia Cells is Associated with Chromatin and DNA. Mol. Cell Biochem. 151:107-111
15. Gogu, S.R., Beckman, B.S., Wilson, R.B., and Agrawal, K.C. (1996). Zidovudine Induced Inhibition of Erythropoietin Receptor, c-fos Expression, and Protein Kinase C Activity in Elythroid Progenitor Cells: Reversal with a Combination of Erythropoietin and Interleukin-3. Biochem. Pharm. 50:413-419.
16. Scandurro, A.B., Rondon, I.J., Wilson, R.B., Tenenbaum, S.A., Garry, R.F., and Beckman, B.S. (1997). Interation of Erythropoietin RNA Binding Protein with Erythropoietin RNA Requires an Association with Heat Shock Protein 70. Kidney Int. 51:579-584.
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18. Asa, P.B., Wilson, R.B., and Garry, R.F. (2002). Antibodies to Squalene in Recipients of Anthrax Vaccine. Exp. Mol. Path. 73:19-27.
19. VandeVord, P.J., Gupta, N., Wilson, R.B., Vinuya, R.Z., Schaefer, C.J., Canady, A.I., and Wooley, P.H. (2004). Immune Reactions Associated with Silicone-Based Ventriculopereitoneal Shunt Malfunctions in Children. Biomat. 25:3853-3860.
20. Szabo S, Haislip AM, Traina-Dorge V, Costin JM, Crawford BE 2nd, Wilson RB, Garry RF. (2005) Human, rhesus macaque, and feline sequences highly similar to mouse mammary tumor virus sequences. Microsc Res Tech. 68:209-221.
21. Sander DM, Szabo S, Gallaher WR, Deas JE, Thompson JJ, Cao Y, Luo-Zhang H, Liu LG, Colmegna I, Koehler J, Espinoza LR, Alexander SS, Hart DJ, Tom DM, Fermin CD, Jaspan JJ, Kulakosky PC, Tenenbaum SA, Wilson RB, Garry RF.(2005). Involvement of Human Intracisternal A-Type Retroviral Particles in Autoimmunity. Microscopy Research and Technique 66:222-234.
22. Sainz B Jr, Mossel EC, Gallaher WR, Wimley WC, Peters CJ, Wilson RB, Garry RF. (2006). Inhibition of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) infectivity by peptides analogous to the viral spike protein. Virus Res 120:146-155.
23. Bazzichi L, Giacomelli C, De Feo F, Giuliano T, Rossi A, Doveri M, Tani C, Wilson RB, Bombardieri S. (2007). Antipolymer antibody in Italian fibromyalgic patients. Arthritis Res Ther. In press.

C. Research Support

1UC1AI067188-01 Garry (PI) 7/1/05 – 6/30/08 EFFO

NIAID

Recombinant antigen multiagent diagnostic assays for Lassa and other arenaviruses

The goals are to develop modern IgM-, IgG- and antigen-capture assays for diagnosis of infection by Old and New World arenaviruses.

Role: Investigator

No overlap.

1R41AI068230-01 Garry (PI) 7/1/06 – 6/30/08 EFFOR

NIAID

Peptide drugs against influenza virus

Synthetic peptides inhibitors of influenza virus, a virus with a class I viral fusion protein, will be developed and tested in vitro and in vivo.

Role: Investigator

No overlap.

RESOURCES - TUHSC

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the project/performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. If research involving Select Agent(s) will occur at any performance site(s), the biocontainment resources available at each site should be described. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

Drs. Garry and Bausch have general laboratory space totaling >4000 sq. ft. on the Fifth floor of the J. B. Johnson Building, which is attached by two covered walkways to the Tulane Medical School Building. This floor is dedicated entirely to research on infectious diseases. In addition, there is a 2,000 square foot BSL-2+ Laboratory that is being upgraded to BSL-3 requirements. Please refer to the Continuation Pages for details regarding the laboratory infrastructure we have built in West Africa.

Clinical:

Please see continuation pages.

Animal:

The Tulane Medical Center vivarium is a professionally staffed facility housed within the same building as the laboratory space.

Computer:

All involved investigators have personal computers within the laboratories and adjacent office spaces and are connected by an ethernet LAN. Tulane has implemented a modern web based purchasing and grant managing system that is available to staff via the network.

Office:

The Departments of Microbiology and Immunology, and Tropical Medicine provide excellent secretarial assistance for grants management and manuscript preparation. Drs. Garry and Bausch maintain offices (110-200 square ft.) on the same floor of the J. Bennett Johnston Building in close proximity to each other and to their respective laboratories.

Other:

The Biotechnology Core Facility in the Department of Biochemistry at TMC provides excellent peptide and polynucleotide synthesis services. Mass spectrometer/MALDI-TOF services are available for Tulane University faculty at the uptown campus core facility.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

The major equipment available in the Investigators' laboratories includes western blot/electrophoresis equipment, horizontal gel electrophoresis equipment, sequencing gel apparatuses, scanning densitometer, gel dryer, chemical hood, Perkin-Elmer and Hybaid thermocyclers, Sorval RT6000 table top centrifuge, microfuges, Quantum spectrophotometer and plate washers, an ELISA reader, cell culture incubators, a Beckman liquid scintillation counter, and RPLC and HPLC set-ups with UV detectors. Various sizing and ion-exchange chromatography columns for protein purification are also available for these studies. The BSL-3 Laboratory is equipped with Class 2 Biohazard Hoods, a Beckman ultracentrifuge, a Sorval and a Beckman table top centrifuge with containment buckets, inverted microscopes, and three -90° freezers.

Resources cont.
Tulane University resources in West Africa
through the Mano River Union-Lassa Fever Network

Although Lassa fever poses a major public health problem in the MRU countries, civil unrest in the region over the past two decades has impeded research and control. Civil war in Sierra Leone forced closure of a CDC research program on Lassa fever in Sierra Leone in 1993 (Bausch, Sesay, and Oshin, 2004). However, in recent years, new-found peace in West Africa has enabled a capacity building project that presents a new and unique opportunity. In 2004 the Mano River Union Lassa Fever Network (MRU LFN) was established to assist MRU countries in the development of national and regional prevention and control strategies for Lassa fever and other outbreak-prone diseases (Khan et al., 2008) (Fig. 9 in the Experimental Plan). The cornerstone of the MRU-LFN has been to enhance laboratory diagnostic and research capacity. The enhanced physical and organizational infrastructure provided by the MRU-LFN present an unparalleled opportunity to access and test clinical samples in an area where Lassa fever is a frequent occurrence.

Fig A. Sample Processing in the Kenema Government Hospital Lassa Laboratory. Samples are manipulated in class II biosafety cabinets by personnel wearing full personnel protective materials.

The MRU-LFN is sponsored by the BioRisk Reduction for Dangerous Pathogens team of WHO's Department of Epidemic and Pandemic Alert and Response (CDS/EPR), with Tulane University contracted as the principle implementing partner. Various investigators on this proposal play integral roles in the MRU-LFN, including Dr. Bausch, who serves as the MRU-LFN director, and Dr. Guttieri, whose laboratory at USAMRIID is the supplier of diagnostic reagents. The MRU-LFN has received financial and technical support from a diverse group of organizations, including MRU country governments, WHO, Tulane, the United Nations, NIH, the United States Agency for International Development (Office of Foreign Disaster Assistance) and USAMRIID. The necessary administrative infrastructure for research in the MRU countries has also been established; all three countries have ethics committee to review and approve research protocols and have received Federal Wide Assurances from the U.S. Department of Health and Human Services.

Clinical

The initial focus of the MRU-LFN has been Kenema District in eastern Sierra Leone, which has the highest incidence of Lassa fever in the world (Fraser et al., 1974; McCormick, 1987). Kenema Government Hospital (KGH), a the 350-bed referral hospital for the region, maintains a year-round 25-bed ward for the care of patients with Lassa Fever at which up to 200 suspected cases may be seen yearly (Bausch, Sesay, and Oshin, 2004; Khan et al., 2008). To our knowledge, this is the only facility continuously operated and dedicated to the care of patients infected with a Category A Select Agent anywhere in the world. The ward is staffed with a full-time team of doctors, nurses, and technicians. An Outreach Team also exists, whose job it is to identify suspected cases of Lassa fever and trace contacts in the community. The Lassa fever ward, staff, patient care and outreach activities are supported by the Sierra Leone Ministry of Health and Sanitation, with additional support from Tulane. The European Union recently funded the MRU-LFN to enhance surveillance for Lassa fever and other epidemic-prone viral diseases in eastern Sierra Leone, which should further augment the detection of cases, as well as the number of clinical samples available for testing and research.

Fig. B. The Kenema Government Hospital Laboratory. The laboratory is comprised of an approximately 5,500 square foot building divided into a general clinical laboratory for routine diagnostics and a 700 square-foot specialized biosafety level-3 suite for manipulation of samples from suspected cases of Lassa fever. Three rectangular solar panels that can faintly be seen on the roof provide essential power for refrigerators, freezers and other essential instruments. The existing Kenema Government Hospital Lassa Ward is behind the buildings up the hill to the left, not visible in this view.

In addition to Kenema, there are presently three other laboratories in the MRU-LFN from which may supply samples for the project: the *International Center for Research on Tropical Infections* (French acronym: CIRIT) and the *Program on Hemorrhagic Fevers* (French acronym: PFHG) in Guinea; and the *Central Public Health Laboratory* (CPHL) in Liberia (Fig. 9 in the Experimental Plan). These laboratories are in various stages of development. Tulane University has supported and engaged with CIRIT on various Lassa fever projects since 1996 (Bausch et al., 2001; Bausch et al., 2000; Demby et al., 2001; Fair et al., 2007). The Tulane program is an extension of the now-concluded CDC *Guinea Lassa Fever Research Program* that Dr. Bausch established while director of the CDC's LF activities in West Africa from 1996-2002. The CIRIT laboratory is on the grounds of N'Zérékoré Regional Hospital, a 200-bed regional reference center with a broad catchment area in the heart of the endemic zone for Lassa fever in the Guinea Forest Region. The Tulane program also includes links with Faranah Hospital in the center of the Lassa fever -endemic region of the savannah. Although PFHG, based in the capital city Conakry, is not in an endemic area for Lassa fever, samples are often funneled through the laboratory, which has an active research program on Lassa fever with support from various European partners (Fichet-Calvet et al., 2008; Fichet-Calvet et al., 2007; ter Meulen, 1999; ter Meulen, 2000; ter Meulen et al., 2001; Ter Meulen et al., 1996). CPHL in Monrovia is the reference laboratory for the country of Liberia, collecting samples from throughout the country.

Fig. C. The structure shown, which is adjacient to the KGH laboratory, is constructed from prefabricated office containers donated by the United Nations, some of which have been subsequently refitted to serve as PCR clean rooms. A 2006 Toyota Land Cruiser is available to support the proposed project.

Laboratory

A laboratory has been established in Kenema for diagnostics for Lassa fever and other viral diseases. Samples are manipulated in class II biosafety cabinets by personnel wearing full personnel protective materials (gowns, gloves, and mask) (Fig. A). The KGH Lassa Laboratory

Fig. D. Class II biosafety cabinets in the KGH laboratory.

is located on the grounds of the hospital, but in a stand-alone building constructed in 2005 (Fig. B). Testing is performed in a specialized BSL-3 suite for manipulation of samples from suspected cases of Lassa fever. ELISA (antigen and IgM and IgG antibody) and real-time PCR for Lassa fever are performed following published protocols (Bausch et al., 2000; Drosten et al., 2002; Drosten et al., 2003) with some modifications. LASV antigens, monoclonal antibodies, and other reagents for ELISA are provided by USAMRIID. In March 2008 the KGH Lassa Laboratory underwent site review by NIH Program Officers and was found acceptable.

The building has approximately 5,500 square feet of laboratory space divided into a general clinical laboratory for routine diagnostics and a 700 square-foot specialized BSL-3 suite for manipulation of samples from suspected cases of Lassa fever. Access to the building and the BSL-3 suite is controlled and recorded. Negative airflow is maintained. The laboratory possesses equipment and trained personnel for diagnostics using real-time PCR (with separate PCR suites – Fig. C), ELISA, and immunofluorescent antibody tests. For safety reasons, no cell culture is performed. The building is equipped with redundant power sources, including town power in Kenema (which is extremely sporadic) and 100 and 6 kilovolt generators, the former used to power the entire laboratory and the latter essential equipment only. The cold storage consists of a -80°C freezer connected to the town power/generator grid and solar powered -20°C freezers and refrigerators. Established biosafety and biosecurity guidelines are maintained, with oversight by WHO and Tulane through the MRU-LFN.

Equipment

Pertinent items in the KGB laboratory include 2 class II biosafety cabinets (Fig. D), light-cycler real-time PCR machine, ELISA plate readers and washers, fluorescent microscope, conventional microscope, non-CO_2 incubator, centrifuge, CD4 counter, water bath, instrument sterilizer, solar and electric powered freezer (Fig. E), refrigerator, water purification system, pH meter, vortex, balances and vacuum pumps. A 2006 Toyota Land Cruiser, essential for Program activities, is well maintained and available for field studies (Fig. C).

Fig. E. Solar powered freezer in the KGH laboratory.

At present, the KGH Lassa Laboratory is the only laboratory in West Africa with the capacity and quality control to perform diagnostic testing for Lassa fever on human samples. However, the other MRU-LFN laboratories are accustomed to receiving samples from suspected cases of Lassa fever, safely packaging them (specific training on this matter has been conducted through the MRU-LFN), and delivering them by ground transport to Kenema for diagnostic testing. For example, almost every other week, samples are sent from Liberia to Kenema, with the diagnostic result based on ELISA and real-time PCR (Fig. F.) communicated by phone within 24 hours. Furthermore, arrangements are presently underway to expand the MRU-LFN to include Nigeria, where Lassa fever is also endemic (and first discovered in 1969). Collectively, the specimens obtained through the MRU-LFN should naturally include a spectrum of antigen and antibody profiles (e.g. high antigenemia from fatal cases, high IgM antibody from recovering cases) with which to validate the commercial

Fig F. Roche LightCycler 2.0 – real-time PCR machine in the KGH laboratory.

assays. Letters of support from the relevant scientists and officials in the MRU countries are provided.

RESOURCES – Autoimmune Technologies

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the project/performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. If research involving Select Agent(s) will occur at any performance site(s), the biocontainment resources available at each site should be described. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

Autoimmune is currently leasing its laboratory space from the Children's Hospital Research Institute for Children of New Orleans (RIC) and has approximately 1260 sq ft of BSL2 laboratory space. Autoimmune also has access to shared equipment rooms and facilities at the RIC.

Clinical:

NA

Animal:

NA

Computer:

All key personnel and technicians have desk top computers and printers.

Office:

Autoimmune's Corporate headquarters is located in New Orleans, Louisiana and consists of approximately 1,000 sq ft. of office space. All personnel on project have fully equipped offices.

Other:

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

BSL-2 laboratory space includes a fume hood, and a 4' Nuaire Class II A2 biosafety cabinet. Autoimmune also owns the following equipment: Applied Biosystems Genamp 9700 PCR workstation; AirCl AirClean 600; 6' Thermo 1400 Class II A2 biosafety cabinet; Nuaire (stacked) 2700 carbon dioxide incubators (13 cu ft total); two Sanyo MCO 20AIC 2700 carbon dioxide incubators (15 cu ft total); BioFlo 110 Modular Fermentor & Bioreactor (14.0 L); microscopes, 2 Nikon Diaphots with ELWD phase phase-contrast condensers and phase phase-contrast 4,10,20 and 40 x objectives; Matrix WellMate; barcoding scanners; BioRad BioLogic LP, low pressure liquid chromatograpy system with fraction collector; high high-purity nitrogen regulator; Thermo 8600 ultralow ((-86 C) freezer; refrigerated microcentrifuge, IEC, Micromax RF; portable microcentrifuge; Eppendorf table-top refrigerated centrifuge, IEC CL31R with large large-capacity swing swing-out rotor, microcentrifuge tube rotor, both with biocontainment capibility, and a rotor for 50 ml tubes; 3 gravity incubators; Fisher Isotemp; 5 water baths; mixers; rockers; orbital shakers, plates shaker, vortex mixers; acrylamide and agarose gel electrophoresis equipment; balances; pH meter; and an Eppendorf Biophotometer Spectrophotometer.

RESOURCES - Corgenix

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the project/performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. If research involving Select Agent(s) will occur at any performance site(s), the biocontainment resources available at each site should be described. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

The Corgenix facility is 15,000 square feet, with 7,500 square feet of manufacturing and research laboratories, 1,000 square feet for refrigerated finished product storage and shipping and receiving, and an additional 1,000 square feet for quality assurance and regulatory activities.

Clinical:

NA

Animal:

NA

Computer:

Each scientist has a computer and instruments are run with separate computers that are isolated from internet access. All computer access is controlled by a secure server. Daily backup procedures are used to preserve data with automated complete backup of all computers and weekly offsite storage of media.

Office:

Corporate headquarters is located in Westminster, Colorado. All personnel on project have fully equipped offices.

Other:

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

The company has two major pieces of manufacturing equipment used in ELISA product production. The Oyster Bay Microplate Processor is an automated ELISA plate coating system that can process 700 plates per hour. The Dynatech Microplate Processor, a second automated plate coating system, is maintained as back-up equipment for plate processing. Corgenix currently manufactures 30,000 ELISA microplates per year and has the capacity to manufacture approximately 100,000 without the addition of new equipment. The company has capabilities in protein purification, conjugation, bulk solution preparation, semi-automated liquid component filling, in-house label design and production, and product assembly. There is back-up equipment for all critical steps in the manufacturing process for ELISA Test Kit production. Corgenix has all necessary equipment for lateral flow assay development, but is requesting funds to upgrade their instrumentation for applying consistent amounts of liquid reagents to the flow membrane and reagent pads.

RESOURCES - Vybion

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the project/performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. If research involving Select Agent(s) will occur at any performance site(s), the biocontainment resources available at each site should be described. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

Vybion laboratories are contained within approximately 6,500 SF under GLP compliant laboratories. Separate labs include molecular biology, fermentation, cell culture, process development and purification and analytical.

Clinical:

NA

Animal:

NA

Computer:

Each scientist has a computer and instruments are run with separate computers that are isolated from internet access. Acomputer access is controlled by a secure server. Daily backup procedures are used to preserve data with automated complete backup of all computers and weekly offsite storage of media.

Office:

There is approximately 1,000 SF of office space including scientist offices, administration, management, conference room, break room.

Other:

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Four BSL2 laminar flow hoods, 45 Cubic Ft of CO2 incubators, 3 inverted microscope (fluoresence), roller bottle and spinner flask capability, two Bioflow 3000 15L fermentors with computer control on DO, temperature, 4 feeds, rpm, pH with full report capabilities, one extra fermentor tank for microbial/yeast and one of cell culture, four akta primes for medium scale purification, one AKTA explorer for development and pilot scale with adapter kit (all with independent computers), two quad pump HPLCs with column condition control, UV/Vis and PDA detection, autosamplers, four buffer blend capabilities and GMP compliant computer software, fluorescence and UV/vis plate readers, spectrophotometer, capillary electrophoresis with autosampler and full computer document and data package, PCR machines, luminomoter, three small and lone arge high speed high capacity centrifuges, five SDS PAGE tanks/WB transfers, microfluidizer, dynomill, sample extration, concentration, four Tangential flow systems up to 200L/hr capacity, numerous balances, two autoclaves, numerous pumps for filtration and TF systems and standard table top equipment. DI water system with routine filter change out and endotoxin monitoring. Access to sequence facility with 3750 sequencers, LCMS Maldi TOF and electrospray at Cornell University. Vybion has two 14L NBS fermentors with extra tank set ups for cell culture applications in addition to over 20L capacity for cell culture flasks and/or roller. Other equipment includes multiple table top and floor high speed centrifuges with up to 12L capacity, microfluidizer, Akta Explorer and Alta primes, filtration housings, 200 L refold tanks, CE, and other equipment necessary for a typical preclinical and scale up operation. Vybion's quality assurance includes GLP level programs and monitoring.

RESOURCES - USAMRIID

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the project/performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. If research involving Select Agent(s) will occur at any performance site(s), the biocontainment resources available at each site should be described. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

Dr. Guttieri has access to BSL-2, BSL-3, and BSL-4 laboratories at USAMRIID. These laboratories are fully equipped and operational. Support includes personnel in the Division of Veterinary Medicine, Division of Pathology, and Aerobiology Division.

Clinical:

NA

Animal:

USAMRIID has a complete array of animal facilities ranging from BSL-2 holding areas to BSL-4 animal rooms. Animals are cared for by personnel in the Division of Veterinary Medicine.

Computer:

Dr. Guttieri has desk top and lap top computers, printers, digital scanners, and digital camera. All key personnel and technicians have desk top computers and printers. USAMRIID's office of Biometrics supports all information technology

Office:

All personnel on project have fully equipped offices.

Other:

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Dr. Guttieri has all equipment required for the proposed research. Speciality equipment required for the proposed animal studies include hematology analyzer, blood chemistry analyzer, pulse oximeter, and oxymax respiration test chamber system. Dr. Guttieri also has access to fluorescent, confocal, and electron microscopes. USAMRIID also has a gamma source for inactivating pathogens.

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert, F.

CHECKLIST

TYPE OF APPLICATION *(Check all that apply.)*

☒ NEW application. *(This application is being submitted to the PHS for the first time.)*

☐ REPLACEMENT of application number:
(This application replaces a prior unfunded version of a new, renewal, or revision application.)

☐ RENEWAL of grant number:
(This application is to extend a funded grant beyond its current project period.)

☐ REVISION to grant number:
(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of program director/principal investigator.

Name of former program director/principal investigator:

☐ CHANGE of Grantee Institution. Name of former institution:

☐ FOREIGN application ☐ Domestic Grant with foreign involvement List Country(ies) Involved:

INVENTIONS AND PATENTS *(Renewal appl. only)* ☐ No ☐ Yes

If "Yes," ☐ Previously reported ☐ Not previously reported

1. PROGRAM INCOME ***(See instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)

2. ASSURANCES/CERTIFICATIONS ***(See Instructions.)***
In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

3. FACILITIES AND ADMINSTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

☒ DHHS Agreement dated: 04/04/08 ☐ No Facilities And Administrative Costs Requested.

☐ DHHS Agreement being negotiated with Regional Office.

☐ No DHHS Agreement, but rate established with Date

CALCULATION* *(The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)*

a. Initial budget period:	Amount of base $	475,418	x Rate applied	49	% = F&A costs	$	232,955
b. 02 year	Amount of base $	382,182	x Rate applied	49	% = F&A costs	$	187,269
c. 03 year	Amount of base $	389,151	x Rate applied	49	% = F&A costs	$	190,684
d. 04 year	Amount of base $	396,323	x Rate applied	49	% = F&A costs	$	194,198
e. 05 year	Amount of base $	403,712	x Rate applied	49	% = F&A costs	$	197,819
					TOTAL F&A Costs	$	1,002,925

*Check appropriate box(es):

wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.):*

4. DISCLOSURE PERMISSION STATEMENT: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)? ☒ Yes ☐ No

TITLE: Preclinical development of recombinant antigen diagnostics for Lassa fever

SPECIFIC AIMS (MILESTONES)

This project will develop **Diagnostics For Biodefense** against Lassa fever, a severe, often fatal viral hemorrhagic fever (VHF). Because of its high case fatality rate, ability to spread easily by human-human contact, and potential for aerosol release, Lassa virus (LASV), the causative agent of Lassa fever, is classified as a Biosafety Level 4 and NIAID Biodefense category A agent. The potential use of LASV as a biological weapon directed against civilian or military targets necessitates development of *"effective, rapid, highly sensitive, specific, easy to use, adaptable, and cost-effective medical diagnostics for public health laboratories, hospital-based clinical laboratories, and point-of-care use (RFA-AI-08-001)"* to diagnose individuals exposed to and/or infected with LASV. The impact of Lassa fever in endemic areas of West Africa is immense, and a safe and effective diagnostic can also provide a very significant public health benefit. Our team has successfully produced prototype LASV enzyme-linked immunosorbent assays (ELISA) that are based on recombinant proteins rather than on reagents that must be produced in high containment laboratories. We have also newly established a research program in Sierra Leone, an area endemic for LASV, that provides unique clinical and laboratory resources for VHF research. We will now perform critical steps in the preclinical development of commercial recombinant antigen Lassa fever diagnostics. This project will process in a manner strictly dependent on timely achievement of the following five milestones:

MILESTONE 1: Development of commercial Lassa fever recombinant ELISA.
1A. Optimize commercial grade recombinant LASV antigen-capture and immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody-capture ELISA using a Performance Panel of well-characterized sera.

MILESTONE 2: Development of Lassa fever recombinant lateral flow point-of-care diagnostics.
2A. Develop LASV recombinant antigen-, IgM- and IgG-capture lateral flow assays.
2B. Optimize the recombinant lateral flow assays as point-of-care diagnostics using a Performance Panel of well-characterized sera.

MILESTONE 3: Manufacturing of Lassa fever recombinant ELISA and lateral flow assays under GMP.
3A. Convert to manufacturing of recombinant ELISA and lateral flow assays under Good Manufacturing Practices (GMP) with Quality Assurance (QA)/Quality Control (QC) to provide quantities of commercial grade diagnostic kits sufficient for preclinical evaluation of design control parameters to achieve benchmarks required for FDA approval.

MILESTONE 4: Process development for production of recombinant LASV proteins and LASV-specific monoclonal antibodies (mAbs).
4A. Optimize scale up/purification of recombinant LASV proteins, GP1, GP2, and NP.
4B. Optimize scale up/purification of mAbs to recombinant LASV GP1, GP2, and NP.
4C.Convert to manufacturing with QA/QC, to provide quantities of recombinant proteins and mAbs sufficient for development and testing of commercial assays.

MILESTONE 5: Field testing of commercial recombinant Lassa fever ELISA and lateral flow point-of-care diagnostics in West Africa.
5A. Define and collect positive and negative sera for assay validation from diverse regions across the Lassa fever endemic range of West Africa and elsewhere.
5B. Test and compare the commercial Lassa fever recombinant ELISA and recombinant lateral flow point-of-care diagnostic assays with results from BSL-4 ELISA and PCR assays.

BACKGROUND AND SIGNIFICANCE

Viral hemorrhagic fevers (VHFs) are serious, often fatal, illnesses characterized by high fever, damage to the vascular system and multi-organ failure. Because of their high case fatality rates, ability to spread easily by human-human contact and potential for deliberate release, many VHF agents are classified in Biosafety Level 4 and NIAID Biodefense category A. VHFs are caused by enveloped RNA viruses of four families: *Arenaviridae*, *Filoviridae*, *Bunyaviridae*, and *Flaviviridae*. All VHF agents have animal or insect natural reservoirs. The occurrence of outbreaks of most VHF in human populations cannot be easily predicted, which offers many challenges for studies that seek to develop diagnostics, therapeutics or vaccines. In contrast to other VHF, in which human cases or outbreaks of hemorrhagic fevers occur sporadically, parts of Sierra Leone, Guinea, Nigeria, and Liberia are endemic for Lassa fever. Our team has newly established a research program in Sierra Leone, which has the highest incidence of Lassa fever, providing unique clinical and laboratory resources for future development of countermeasures against this important VHF. Because it is likely that VHF share many pathogenic mechanisms, work on Lassa fever can guide strategies for the future development of diagnostics, therapeutics, and vaccines against other VHF.

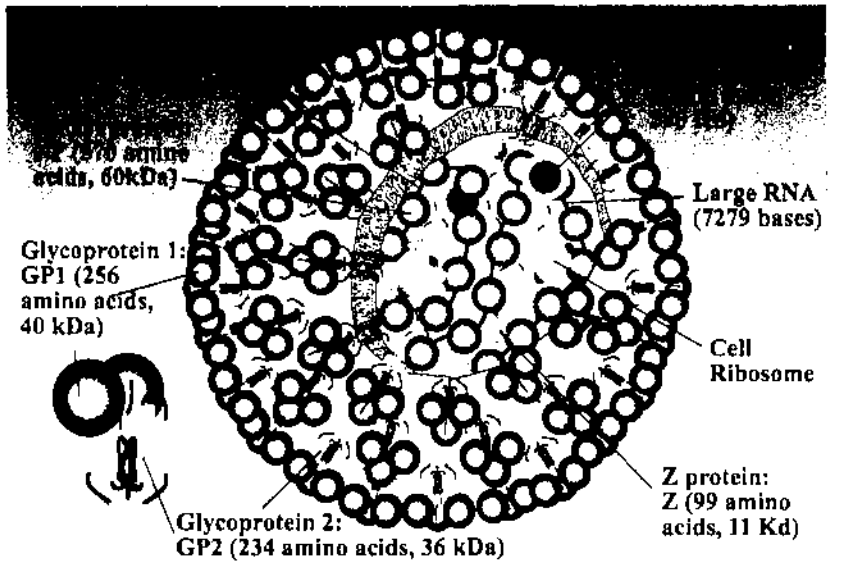

Fig. 1. Lassa virus virion.

Current diagnostic assays for Lassa fever require viral antigens that must be produced in BSL-4 laboratories, making them scarce, expensive and non-commercializable. The current lack of widely available diagnostic tests has precluded accurate surveillance. Nevertheless, it is apparent that the public health impact of Lassa fever in endemic areas is immense (Birmingham and Kenyon, 2001). It has been estimated that there are up to 300,000 cases of Lassa fever per year in West Africa and 5,000 deaths (Fisher-Hoch and McCormick, 2004; McCormick et al., 1987; McCormick et al., 1986). In some parts of Sierra Leone 10-15% of all patients admitted to hospitals have Lassa fever, which places a tremendous burden on an already fragile health care infrastructure. Case fatality rates for Lassa fever are typically 15% to 20%, although in epidemics overall mortality can be much higher (>45%). The mortality rate for women in the last month of pregnancy is always high, ~90%; Lassa fever causes high rates of fetal death at all stages of gestation. Mortality rates for Lassa fever appear to be higher in non-Africans, which is of concern because Lassa fever is the most commonly exported VHF (Haas et al., 2003; Holmes et al., 1990). The proposed project will fill a critical biodefense and public health gap by developing modern diagnostic assays for Lassa fever for commercial production not requiring high containment laboratories.

Lassa virus. The causative agent of Lassa fever is Lassa virus (LASV), a member of the *Arenaviridae* (Murphy, 1975). The genome of arenaviruses consists of two segments (Large, L and Small, S) of single-stranded, ambisense RNA. The enveloped virions (diameter: 110-130 nm) contain two glycoproteins GP1 and GP2 (expressed from a precursor GPC), a single nucleoprotein NP and a ring finger (matrix) protein Z. (Fig. 1). Electron micrographs of arenaviruses show grainy particles that are ribosomes acquired from the host cells (Murphy and Whitfield, 1975). Hence, use of the Latin "arena," which means "sandy" for the family name. The arenaviruses are divided into two groups, the Old World or lymphocytic choriomeningitis virus

(LCMV)/LASV complex and the New World or Tacaribe complex (Bowen, Peters, and Nichol, 1997; Rojek et al., 2008). Other arenaviruses that cause illness in humans include Junin virus (Argentine hemorrhagic fever, AHF), Machupo virus (Bolivian HF), Guanarito virus (Venezuelan HF) and Sabiá virus (Brazilian HF).

Arenaviruses are zoonotic; each virus is associated with a specific species of rodent (Bowen, Peters, and Nichol, 1997). The reservoir of LASV is the "multimammate rat" of the genus *Mastomys* (Bonner et al., 2007; Fichet-Calvet et al., 2008; Fichet-Calvet et al., 2007; Lecompte et al., 2006; Okoror et al., 2005). *Mastomys* show no symptoms of LASV infection, but shed the virus in saliva, urine and feces. The wide distribution of *Mastomys* in Africa makes eradication of this rodent reservoir impractical and ecologically undesirable (Demby et al., 2001). *Mastomys* often live in human homes, and the virus is easily transmitted to humans (Bausch et al., 2001; Demby et al., 2001). Transmission occurs via direct contact with rat urine, feces, and saliva, or by contact or ingestion of excretion-contaminated materials. Infection may also occur when *Mastomys* are caught and prepared as food. LASV is readily transmitted between humans, via exposure to blood or body fluids, which makes nosocomial infection a great concern. Infection usually occurs via mucous membranes or skin breaks. LASV can also be transmitted to sexual partners of convalescent men via semen up to 6 weeks post-infection. The stability of the virus in aerosol, plus the ability of the virus to infect guinea pigs and monkeys via the respiratory route emphasize the potential for LASV aerosol transmission in bioterrorism or other settings (Stephenson, Larson, and Dominik, 1984).

Lassa fever. Signs and symptoms of Lassa fever, which occur 1-3 weeks after virus exposure, are highly variable, but can include fever, retrosternal, back or abdominal pain, sore throat, cough, vomiting, diarrhea, conjunctival injection, and facial swelling (Knobloch et al., 1980; McCormick and Fisher-Hoch, 2002; Richmond and Baglole, 2003). LASV infects endothelial cells, resulting in increased capillary permeability, diminished effective circulating volume, shock, and multi-organ system failure (Peters et al., 1989). Frank bleeding, usually mucosal (gums, etc.), occurs in less than a third of cases, but confers a poor prognosis. Neurological problems have also been described, including hearing loss, tremors, and encephalitis. Temporary or permanent unilateral or bilateral deafness occurs in ~30% of Lassa fever patients, and is not associated with the severity of the acute disease (Cummins et al., 1990; Rybak, 1990). Patients who survive begin to defervesce 2-3 weeks after onset of the disease.

The antiviral drug ribavirin is effective in the treatment of Lassa fever if administered early in the course of illness (Johnson et al., 1987; McCormick et al., 1986). Ribavirin administered to patients with a high virus load (and therefore a high risk for mortality) within the first six days of illness reduced the case-fatality rate from 55% to 5% (McCormick et al., 1986). Several anecdotal reports suggest that this drug can also be effective against other arenaviral hemorrhagic fevers (Barry et al., 1995; Kilgore et al., 1997; Weissenbacher et al., 1986a; Weissenbacher et al., 1986b). The efficacy of prophylactic treatments for Lassa fever is unknown, although it has been suggested that people with high-risk exposures be treated with oral ribavirin. Passive transfer of neutralizing antibodies early after infection may also be an effective treatment for Lassa fever and other arenaviral hemorrhagic fevers (Enria et al., 1984; Frame et al., 1984; Jahrling, 1983; Jahrling and Peters, 1984; Jahrling, Peters, and Stephen, 1984; Weissenbacher et al., 1986a). The dependence of effective treatment on early diagnosis provides a strong rationale for improving LASV diagnostics. Furthermore, no LASV vaccine is currently available. Effective immunodiagnostic assays are absolutely essential for development and field-testing of potential Lassa fever vaccines.

Potential for use of LASV as a bioweapon. In addition to high case fatality rates, LASV has many features that enhance its potential as a bioweapon. LASV has a relatively stable virion, does not require passage via insect vectors, and is spread easily by human-to-human contact. As discussed above, LASV may be capable of aerosol spread or other simple means of dispersal. The high prevalence of Lassa fever in West Africa coupled with the ease of travel to and from this area permits easy access to LASV for use as a bioweapon. A cluster of VHF cases in the United States caused by LASV would be a major public health incident. Because febrile illnesses are common, the absence of reliable diagnostic tests would greatly increase the impact of the attack and permit wider dissemination via human-to-human contact. The potential use of LASV as a

biological weapon directed against civilian or military targets necessitates the commercial development of effective diagnostics.

Diagnostic procedures for Lassa fever. This application is based on the premise that, as was the case with advanced generation HIV antibody tests, commercial Lassa fever immunodiagnostic assays using recombinant LASV proteins can be developed with superior sensitivity and specificity compared to currently available noncommercializable assays based on LASV grown under BSL-4 conditions. Our prior results (Bausch et al. 2000) coupled with newly derived Preliminary Results strongly suggest that antigen-capture and IgM-capture immunoassays will provide the most sensitive and specific diagnostic tests for acute Lassa virus infection. We also plan on developing IgG-capture assays that can assist in determining whether a patient is experiencing acute LASV infection or has had a past infection. IgG-capture assays, if more widely available, can also be used in surveillance, ecology and natural history studies of Lassa fever.

This application focuses primarily on the development of immunodiagnostics, rather than reverse transcriptase-polymerase chain reaction (RT-PCR) or other nucleic acid diagnostics. Based on our Preliminary Results, we expect to be able to develop Lassa fever commercializable recombinant ELISA with superior sensitivity to RT-PCR-based assays. LASV viremia is known to be transient, with virus from peripheral blood cleared rapidly by both innate and early acquired immune responses. RT-PCR and other molecular based assays require the presence of viral RNA in the patient sample (Bausch et al. 2000 and Preliminary Results). The levels of LASV RNA and antigen (virions) in the blood drop rapidly with the appearance of LASV-specific IgM antibodies. In Preliminary Results we observed poor performance of PCR based assays compared to immunoassays such as Ag-capture. While Ag-capture also requires the presence of virions or protein in the blood, a possible explanation for the relatively poor PCR performance is that the current strains of LASV circulating in Sierra Leone have diverged from the prototype Josiah strain, which could affect PCR primer binding, PCR sensitivity and perhaps virulence. The diversity amongst LASV isolates suggests that it may be impractical to develop useful RT-PCR strategies for rapid detection (Archer and Rico-Hesse, 2002; Bowen, Peters, and Nichol, 1997; Niedrig et al., 2004). Indeed, the International Quality Assurance Study on the Rapid Detection of Viral Agents of Bioterrorism by RT-PCR methods found that only a fraction (21-50%) of established biodefense laboratories could detect common strains of LASV in samples containing fewer than 5000 copies/ml (Niedrig et al., 2004). More recent studies have attempted to improve on these results, but these RT-PCR-based assays have not been tested on large numbers of samples from diverse areas across the endemic range of LASV (Vieth et al., 2007).

An important reason to consider immunoassays over PCR methods is that the latter require instrumentation, expertise and facilities generally not available in LASV endemic areas of West Africa (Demby et al., 1994; Lunkenheimer, Hufert, and Schmitz, 1990; Trappier et al., 1993). Additional advantages of ELISA-based diagnostics include their ease of standardization and use in comparison to PCR-based assays. It should be possible to combine LASV detection with detection for selected pathogens that have a clinical presentation similar to Lassa fever, such as Ebola virus or dengue virus. This application will also take advantage of the fact that ELISA can be converted to formats, such as lateral flow, that can be especially valuable for rapid diagnosis during an incident of bioterrrorism and could also be used in the technology poor regions of West Africa. This is particularly important for health facilities in developing countries where Lassa fever is endemic and where the number of patients that fit the differential diagnosis of this disease can be considerable. Unfortunately, these facilities often lack basic laboratory facilities to perform required laboratory tests to confirm the diagnosis. The possibility of having laboratory tests done quickly and results available immediately is essential for Lassa fever, a severe and life-threatening disease that requires prompt medical intervention with ribavirin. A lateral flow assay is ideal for use as a point-of-care test in health care centers that lack the expertise and facilities to perform more complex assays, and could also greatly improve response times in the event of LASV deliberate release.

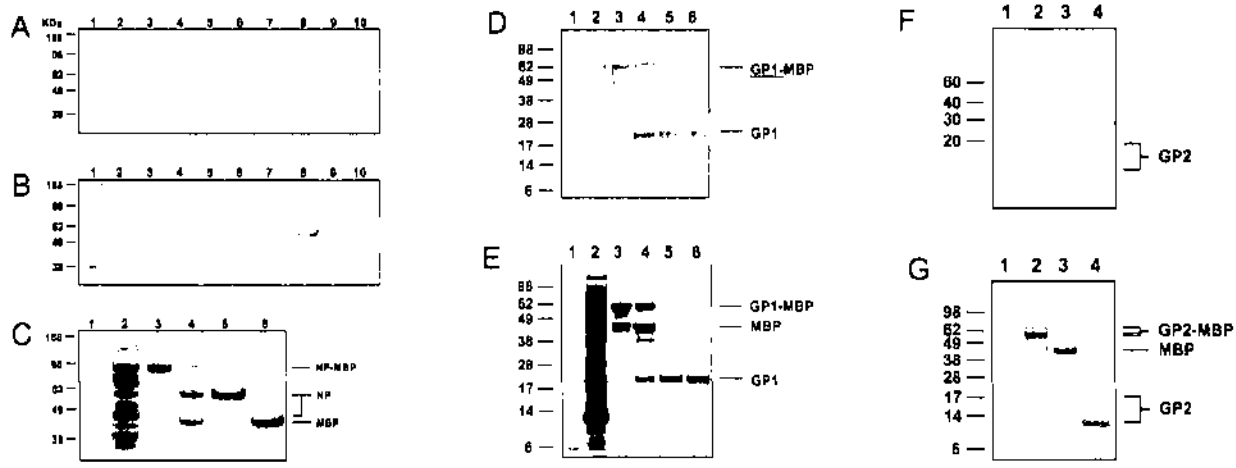

Fig. 2. Bacterial expression of LASV proteins. A: Expression and purification of LASV NP from *E. coli* Rosetta 2(DE3) cells transformed with construct pMAL-c2x:NP. Western blot: lane 1 in this and subsequent panels is XP molecular weight markers (kDa). Lane 2: amylose capture eluate, lane 3: Factor Xa cleavage reaction, and lanes 4-10: SEC fractions 4-10. The blot was probed with a rabbit γ-MBP polyclonal antibody and then detected with an HRP-conjugated goat γ-rabbit IgG antibody. B: The Western blot in panel A was stripped, reprobed with LASV mAb mix containing mAbs to NP, GP1 and GP2. C: SDS-PAGE and Coomassie blue stain of proteins. Lane 2: whole bacterial cell lysate, lane 3: amylose capture eluate, lane 4: Factor Xa cleavage reaction, lane 5: SEC-purfied NP generated from pooled NP-containing fractions, and lane 6: SEC-purified MBP. D: Expression and purification of LASV GP1 from *E. coli* Rosetta gami 2 cells transformed with construct pMAL-c2x:GP1. Western blot: Lane 2: whole bacterial cell lysate, lane 3: amylose capture eluate, lane 4: Factor Xa cleavage reaction, lanes 5 and 6: SEC-purified GP1 generated from pooled GP1-containing fractions. The blot was probed as in B. F: Expression and purification of LASV GP2 from *E. coli* Rosetta gami 2 cells transformed with construct pMAL-c2x:GP2. Western blot: Lane 2: amylose capture eluate, lane 3: Factor Xa cleavage, and lane 4: pooled SEC fractions. The blot was probed as in B. E and G: SDS-PAGE and Coomassie blue stain of proteins in D and F respectively. From Branco *et al.*, 2008.

PRELIMINARY RESULTS

This project unites a uniquely experienced group of researchers from academia, industry and the United States military. The combined expertise, demonstrated in part by Preliminary Results presented here, is essential to develop commercial recombinant antigen LASV diagnostics that will fulfill vital needs for biodefense and public health. The proposed project builds on extensive work by members of this investigative team that have developed unique clinical and research facilities for the study of Lassa fever in post Civil War Sierra Leone and other countries in West Africa.

Diagnosis of Lassa Fever. In West Africa where LASV is endemic, early manifestations of Lassa fever are often indistinguishable from those of many other febrile illnesses such as malaria or typhoid (McCormick et al., 1987). It is not unusual for a patient to assume that LASV infection is a severe episode of malaria. Similar difficulties would be encountered in the United States where febrile illnesses are common, but few physicians have ever directly encountered a VHF patient. In the case of Lassa fever, early treatment with ribavirin and/or passive transfer of neutralizing antibodies can dramatically reduce mortality (Enria et al., 1984; Frame et al., 1984; Jahrling, 1983; Jahrling and Peters, 1984; Jahrling, Peters, and Stephen, 1984; McCormick et al., 1986; Weissenbacher et al., 1986a). Thus, prompt identification of infected individuals is crucial, either in the case of deliberate attack or in endemic areas (Fisher-Hoch et al., 1995). Formerly, laboratory diagnosis of acute LASV infection in West Africa employed the indirect fluorescent-antibody (IFA)

test (McCormick et al., 1987; Wulff and Lange, 1975). However, IFA lacks sensitivity and specificity relative to modern diagnostic assays (Van der Waals et al., 1986). Previously, the current generation of LASV antigen-capture ELISA and IgM- and IgG-capture ELISA, which are based on reagents derived from BSL-4 grown LASV, were evaluated (Bausch et al., 2000). Samples were field-collected from 305 West African patients suspected of having acute LASV infection. In those patients confirmed to have Lassa fever, LASV antigen typically appeared in the first week of illness, to be subsequently replaced by IgM in the second week. LASV-specific IgM typically remained positive through the last drawn sample taken at a mean of 16.4 days (range, 4 to 40 days) after symptom onset. LASV-specific IgG began to appear around week 3. The IgM-capture ELISA proved to be the single most sensitive BSL-4 reagent based assay in detecting acute infection overall, identifying 36 (72%) of 50 confirmed cases from which LASV could be isolated (Bausch et al., 2000). In practice, LASV Ag- and IgM-capture ELISA are used in tandem. Using current WHO criteria, combining both confirmed and suspected Lassa fever cases, the sensitivity and specificity of the BSL-4 ELISA Ag/IgM was 92% and 99%, with positive and negative predictive values of 96% and 97%, respectively. IgG antibody was detected in 50 (16%) of the 305 suspected cases, but in only 9 (18%) of the 50 acute cases of LASV infection. Thus, the presence of IgG antibody suggests previous exposure to LASV.

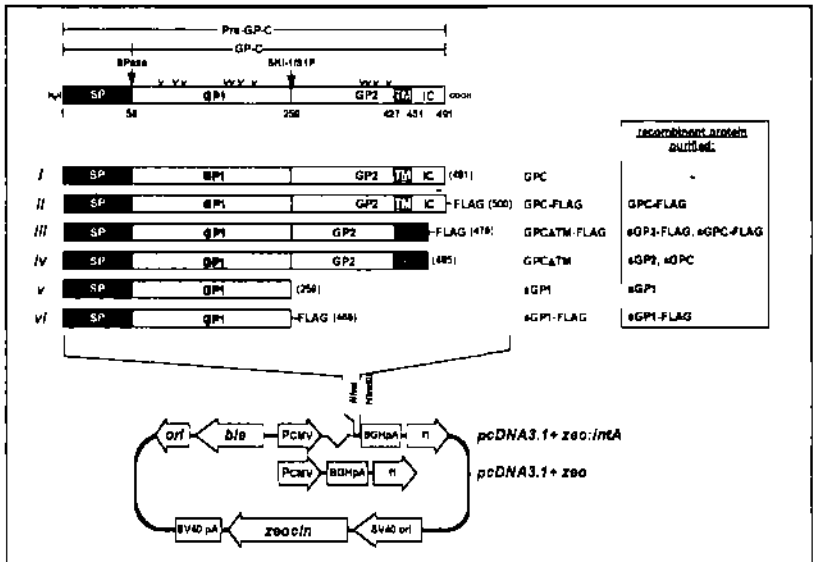

Fig. 3. Cloning strategy for expression of LASV GPC, GP1, and GP2 in mammalian cells using CMV promoter-driven eukaryotic vectors.

Recombinant antigen-based diagnostic assays for LASV and other arenaviruses. Production of current diagnostic tests for Lassa fever requires high containment BSL-4 facilities, using live LASV as the source of capture antigen or for production of antibody reagents (Bausch et al., 2000). Virus isolation or RT-PCR-based assays can also be used to diagnose LASV infection, but such methods are not conducive to field diagnosis and are challenged by the genetic diversity of LASV (**Background and Significance**). BSL-4 and PCR facilities are generally not available in West Africa where LASV is endemic. More importantly, LASV viremia is transient, so only a portion of infected persons presenting at a health care facility will be virus culture, PCR positive or Ag-capture positive. Thus, it is necessary to develop highly sensitive, reliable, simple, and cost-effective diagnostic assays that detect both Ag (or viral RNA) and LASV-specific IgM and can be readily deployed and implemented in the event of a deliberate release, and also be performed in resource-poor settings. Toward these ends, we have reported on the expression, purification and characterization of LASV proteins in bacterial and mammalian cell-based systems and provide evidence that suggests their potential use in the development of a broadly reactive commercializable recombinant diagnostic platform for Lassa fever that includes ELISA and lateral flow point-of-care assays.

Bacterially produced LASV GP1, GP2, and NP. The expression and purification of LASV GP1, GP2, and NP has been achieved in *Escherichia coli* (*E. coli*) through rational combination of fusion protein elements, optimized cytosolic or periplasmic expression environment, increased supply of rare eukaryotic codon tRNAs in the bacterial cell, and fermentation parameters (Fig. 2) (Branco et al., 2008). Full-length NP and the ectodomains of GP1 and GP2 were generated as maltose-binding protein (MBP) fusions in the Rosetta strains of *E. coli* using pMAL-c2x vectors. Average fusion protein yields per liter of culture for MBP-NP, MBP-GP1, and MBP-GP2 were 10 mg, 9 mg, and 9 mg, respectively. Each protein was captured from cell lysates using amylose resin, cleaved with Factor Xa, and purified using size-exclusion chromatography

(SEC). Protein processing and purification steps resulted in average yields per liter of 1.6 mg, 1.5 mg, and 0.7 mg of cleaved and purified NP, GP1 and GP2, respectively. The use of the Rosetta strains of *E. coli* allowed us to rapidly express recombinant LASV proteins for prototype Lassa fever ELISA development. The Rosetta stains, however, have certain limitations that challenge scale-up of recombinant protein production. Therefore, alternative methods for production of LASV proteins will be developed in the current proposal (Milestone 4).

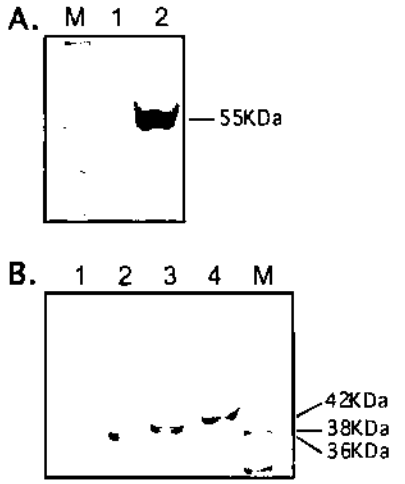

Fig. 4. Mammalian cell-based expression of LASV NP (A), and secreted versions of GP1 and GP2 (B). A: LASV NP was expressed as a HIS-tagged protein in HEK-293T/17 cells and purified from cell extracts 40 hours post-transfection. M: marker; 1: vector only; 2: LASV NP. B: LASV GP1 and GP2 variants were FLAG-tagged and expressed in the same cell background as for NP. Proteins were purified from culture supernatants 72 hours post-transfection. 1: vector only; 2: LASV GPCDTMDIC-FLAG; 3: LASV GPCDTM-FLAG; 4: sGP1-FLAG; M: marker. From Illick et al., in preparation.

Mammalian cell produced LASV GPC, GP1, GP2, and NP. The LASV glycoproteins have been expressed in mammalian systems as native GPC and uncoupled GP1 and GP2 subunits. Constructs expressing GP1 and GP2 were engineered using combinations of promoter elements, signal peptides, transmembrane (TM) and intracellular (IC) domains, and purification tags (Fig. 3). Human cytomegalovirus (hCMV) intron-A greatly enhanced the expression of GPC, GP1, and GP2, in HEK-293T/17 cells using expression vectors driven by the hCMV major immediate early promoter (MIE). Stable NS0 cell lines expressing GPC were generated, whereas soluble (s) GP1, and sGP1-FLAG were expressed transiently in HEK-293T/17 cells and stably expressed in CHO-DG44 cells. Soluble GP1 and sGP1-FLAG are expressed in mammalian cells as glycosylated proteins that migrate on SDS-PAGE gels as homogeneous species in the 42 KDa range. Recent efforts aimed at understanding the requirements for expression of LASV GP2 as a standalone protein, uncoupled from the GPC SP and GP1 have successfully resulted in the generation of secreted GP2. Expression and secretion of GP2 was best achieved when the TM domain of GPC was deleted, but the GP1 ORF including the SKI-1/S1P protease cleavage site, and the IC domain of GP2 remained intact. Soluble GP2-FLAG is expressed and secreted in mammalian cells as a homogeneous species in the 38 KDa range, as determined by SDS-PAGE (Fig. 4B). The secretory GP2-expressing construct will be used to generate stable NS0 and/or CHO-DG44 cell lines. Although LASV NP has been successfully generated and purified as an MBP-fusion protein in *E. coli*, a mammalian cell generated counterpart has been expressed in HEK-293T/17 cells in untagged and 6X-HIS-tagged formats (Fig. 4A). A mammalian cell generated LASV NP protein provides a viable alternative to the bacterial expressed counterpart, particularly if alternative platforms for generation of full-length protein with lowered production costs in prokaryotic systems are not successful. Mammalian cells produced a full length NP-HIS protein that could be easily purified by Ni^{2+} or Co^{2+} metal ion chromatography. The untagged NP protein could be purified by affinity chromatography using anti-NP specific mAbs.

Murine monoclonal antibodies to purified recombinant LASV proteins. A panel of murine monoclonal antibodies (mAbs) has been generated against bacterially generated NP, GP1 and GP2 or mammalian expressed sGP1-FLAG and NS0 cell surface expressed GPC and GP1-TM proteins. To date 128 hybridoma lines have been generated, and most have been cloned by limiting dilution cell cloning (LDCC). A panel of relevant hybridoma cell lines that produce IgGs specific to LASV NP, GP1, and GP2 specificities are being adapted to growth in shaker and spinner flasks using serum free media formulations, for scale-up and streamlined purification of mAbs. Examples of representative mAbs with specificies to various LASV proteins are shown in Table 1.

Table 1. Current status of hybridoma characterization and scale-up for generation of purified mAbs to LASV NP, GP1, and GP2 for assay development

Antigen	designation	Relative binding by ELISA	Relative binding by WB	Isotype	status
NP	692.2L	+++	+++	IgG1	Adaptation to PFHM
NP	1474.13L	+++	+++	IgG1	Adaptation to PFHM
NP	33E	++	+++	IgG1	Adaptation to PFHM
NP	61E	+++	+++	IgG1	Adaptation to PFHM
NP	100E	++	+++	IgG2a	Adaptation to PFHM
GP1	673.12L	+++	+++	IgG2a	Scaling up in PFHM
GP1	910.49L	++	++	IgG3	Scaling up in PFHM
GP1	2335.3L	+++	+++	IgG1	Adaptation to PFHM
GP1	2477.8L	+++	++	IgG1	**mAb in production**
GP2	75.1L	+++	+++	IgG2a	**mAb in production**
GP2	180.1L	+++	+	IgG1	**mAb in production**
GP2	265.2L	+++	+++	IgG1	Adaptation to PFHM
GP2	282.6L	+++	+++	IgG1	**mAb in production**
GP2	706.20L	+++	+++	IgG1	Adaptation to PFHM

PFHM: Protein-Free Hybridoma Medium; WB: Western Blot

Development of prototype recombinant antigen LASV Ag, IgG and IGM ELISA and direct comparison to current assays based on BSL4 grown virus. Prototype LASV Ag-capture and IgM- and IgG-capture ELISA using recombinant LASV proteins and sera or mAbs produced to these recombinant proteins have been developed. We also re-established the BSL-4 IgM capture assay in to enable comparison to the recombinant IgM capture ELISA. This assay was similar to assays previously employed by CDC and USAMRIID except that a rabbit anti-recombinant LASV protein sera was used. Our attempts at reconstituting these BSL-4 IgM capture assays were unsuccessful until we substituted the recombinant rabbit sera into the assays. We believe the problems were due to lack of specificity of available sera prepared after injection of rabbits with disrupted cell culture grown LASV. We also produced an Ag-capture ELISA based on a detection serum from animals immunized with recombinant LASV proteins. In this assay, murine mAbs to LASV proteins are coated onto ELISA plate wells. A 1:10 dilution of patient sera is added to the wells. Then, the detection serum from rabbits immunized with recombinant LASV proteins is added to detect the presence of the LASV antigens in the patient sera. The recombinant ELISA under development have been directly compared for sensitivity and specificity with non-recombinant ELISA and 3 variations of PCR detection (Table 2, Fig. 6). RTqPCR-based assays were established in the diagnostics laboratory at Kenema Government Hospital, Sierra Leone. The three LASV PCR assays used were the USAMRIID and WHO optimized assays, as well as a pan-arenavirus PCR based on primers developed at the Nocht Institute (Hamburg).

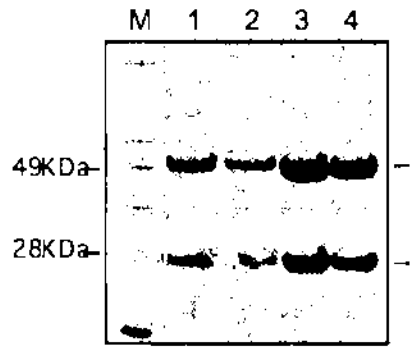

Fig. 5. Purification of mAb from LASV GP2-specific hybridoma cell line 180.2 (IgG1) by Protein A gel chromatography. M: marker; 1: pH5.5 elution; 2: pH4.5 elution; 3: pH3.5 elution; 4: pH2.5 elution. Elution with pH 2.5 or 3.5 generated MAb preparations with purities greater than 95%.

For this field-testing, we initially focused on samples from a serological panel consisting of 96 serum samples from 38 consecutive well-characterized patents attending the Kenema General Hospital Lassa Ward. Current WHO guidelines consider that a patient can only be considered a confirmed Lassa fever case if the Ag-capture assay is positive, but can be considered a "suspected" case, if the IgM assay is positive. Our recombinant Ag- and IgM-capture ELISA under development in this project should permit a revision of these guidelines. For the purposes of the current analysis we considered 26 of the 38 patients that made up our serological panel to be positive for acute LASV infection based on clinical signs and symptoms, recognizing that 7 of these "positive" patients, who were IgM positive only, would technically be classified as suspected cases by current WHO criteria. Twelve patients in the serological panel were considered negative for acute LASV infection based on clinical signs and symptoms. Six of these sign- and symptom-free patients (4 were former patients in the Kenema Lassa Ward) were judged to have had past LASV infections based on negative PCR and Ag-capture, but positive antibody serology.

Table 2. Comparisons of Recombinant LASV ELISA with BSL-4 ELISA and PCR detection using a serological panel from the Kenema Government Hospital Lassa Ward.[1]

	PCR[2]	Ag-capture ELISA	BSL-4 IgM ELISA	Rec. IgM ELISA	Rec IgM + rIgG ELISA	PCR + BSL-4 IgM ELISA	Ag-capture + BSL-4 IgM	Ag-capture plus rIgM	Ag-capture + rIgM/ rIgG
Positive	13	19	11	24	26	20	22	25	26
Negative	11	11	11	12	12	10	11	12	12
False Positive	1	0	1	0	0	2	1	0	0
False Negative	13	8	15	2	0	6	4	1	0
Sensitivity[3]	50.0%	73.1%	42.3%	92.3%	100%	76.9%	84.6%	96.2%	100%
Specificity[4]	91.7%	100%	91.7%	100%	100%	83.3%	91.7%	100%	100%
Efficiency[5]	68.4%	78.9%	57.9%	94.7%	100%	78.9%	86.8%	97.4%	100%

[1]96 serum samples from 38 consecutive well-characterized patents attending the KGH Lassa Ward were analyzed by PCR and various ELISA. 26 patients (Positives) were judged to have acute Lassa infection (WHO confirmed and suspected cases). 12 patients (Negatives) were judged to have either non-Lassa febrile illness (n=6) or to have had past Lassa virus infection (n=6). 4 of these latter patents were follow-up patients from the Lassa ward).
[2]samples were scored positive if positive on one or more of three real-time RT-qPCR assays: USAMRIID standard PCR, WHO alternative PCR or pan-arenavirus PCR.
[3]P/P+FN X 100; [4]N/N+FP X 100; [5]P+N/Total X 100

Comparison of these assays revealed that the recombinant Lassa fever ELISA out-performed the PCR assays and the traditional ELISA, by measures of sensitivity, specificity and efficiency (Table 2). PCR sensitivity for three PCR assays combined was 50% compared to 73% for the Ag-capture assay. Individually, none of the three PCR assays approached 50% sensitivity. The most sensitive was the USAMRIID standard PCR with 31% sensitivity (92% specificity). As described above, this version of the Ag-capture ELISA uses sera from rabbits immunized with recombinant LASV proteins as a detection reagent, and is more sensitive and specific than BSL-4 prior versions of this ELISA. As discussed, both PCR and Ag-capture ELISA require the presence of virions, viral RNA or viral proteins in the patient sample. LASV viremia is known to be transient, with virus from peripheral blood cleared rapidly by both innate and early acquired immune responses. The presumed positive patients in this cohort had a case mortality of 83%, which is higher than expected. Generally, patients coming to the KGH ward (which has only recently been able to perform any LASV testing) had more severe Lassa than in other past surveys. We suspect that they were also further along in the disease course. An alternative, though not mutually exclusive explanation, for

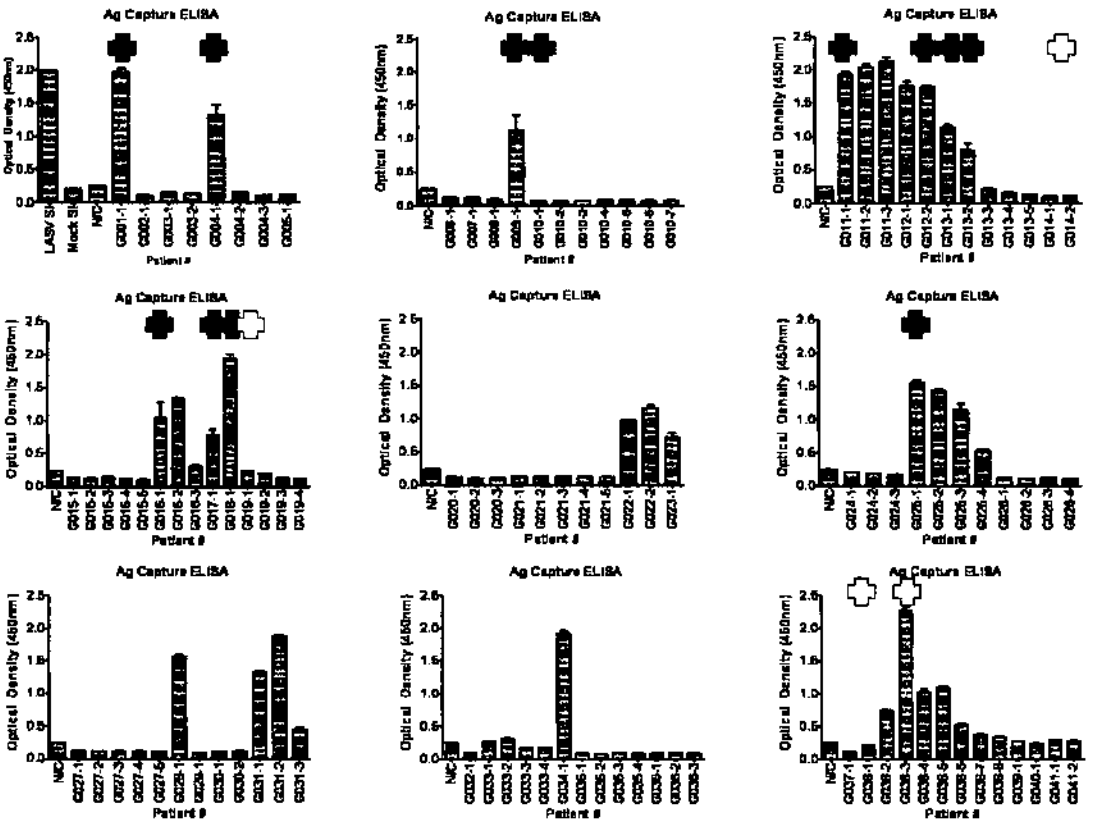

Fig 6. Ag-capture ELISA versus 3 variations of PCR detection. Serum samples from 38 consecutive well-characterized patents attending the KGH Lassa Ward were analyzed by Ag-capture ELISA and three versions of LASV PCR. Results from three additional patients not included in the results of Table 1 are also shown. Red cross: positive sample (WHO confirmed + suspected) was positive on USAMRIID PCR only; Blue cross: positive sample on WHO PCR only; Purple cross: positive sample on Pan-arenavirus PCR only; Green: positive samples on all three PCR; Yellow: Positive sample on WHO and Pan-arenavirus PCR; Orange: Positive sample on USAMRIID and WHO PCR; Black cross: Negative sample false was positive on USAMRIID PCR.

the relatively poor PCR performance is that the current strains of LASV circulating in Sierra Leone have further diverged from the prototype Josiah strain, which could affect PCR primer binding and sensitivity.

The recombinant IgM ELISA was more than twice as sensitive (42 vs. 92%) and more specific (92 vs. 100%) than the BSL-4 IgM ELISA (Table 4), with no false positives. This cohort of patients may be further along in the disease course than in other past surveys. Patients presenting early in the disease course, while viremic, but before the development of an antibody response, would be expected to be negative in any IgG- and IgM-capture ELISA. Therefore, further development of the Ag-capture ELISA will be essential to detect such early Lassa patients, prior to the antibody response to the virus. It should also be noted that a subset of the acute Lassa patients (2/26) did not present with a detectable IgM response, but rather an IgG only response. This has been noted before (Bausch et al, 2000). The practical aspect of this observation is that IgG capture will be used in combination with IgM capture for acute diagnosis of Lassa in addition to being an important assay for LASV surveillance. As expected, antibody responses typically followed PCR or antigen-positivity. There were, however, patients in which LASV RNA (PCR) or Ag and LASV-specific IgM or IgG were detected in the same sample, which has been observed only rarely in past surveys with BSL-4 ELISA. This situation reflects the increased sensitivity of the newly developed recombinant assays compared to the BSL-4 assays illustrated further with a representative patient for which serial serum samples were obtained (Fig. 7).

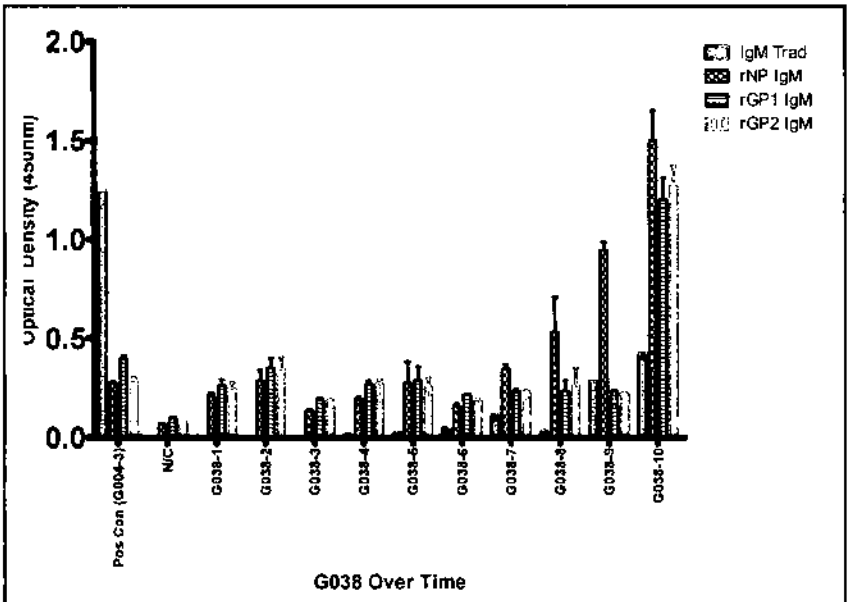

Fig 7. The recombinant Lassa fever IgM-capture ELISA was positive on 24/26 positive (WHO confirmed + suspected) samples, whereas the BSL-4 IgM capture ELISA was positive on 11/26 with 1 one false positive. ELISA configured with recombinant NP, GP1 or GP2 generally were positive earlier and with higher signal than the BSL-4 ELISA as illustrated by serial samples collected from patient G038.

Prior studies indicated that combining the Ag-capture ELISA with the IgM capture ELISA will be the most sensitive and specific method for diagnosing acute Lassa. Indeed, our results combining the Ag-capture ELISA with the BSL-4 IgM capture give sensitivities and specificities surveys (Table 2) that were similar to those of the most extensive previous study (Bausch et al., 2000). However, combining a recombinant Ag-capture with a recombinant IgG/IgM capture assay may provide the most sensitive and specific method for diagnosing acute Lassa fever. Another important advantage is that the recombinant assays are scalable and commercializable, two important features that will be advanced with the current project. PCR did not perform as well in combination with the BSL-4 IgM-capture ELISA. Therefore, we cannot at this time recommend the current USAMRIID PCR or any combination of these PCRs as a substitute for the Ag-capture assay for diagnosing patients in the early stages of Lassa.

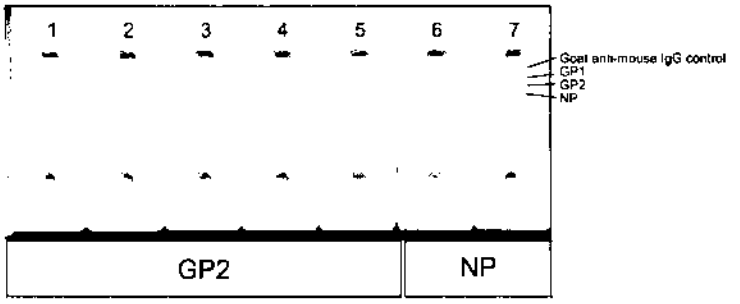

Fig. 8. Monoclonal antibody binding to recombinant LASV proteins in lateral flow assay. Bacterial GP1, GP2 and NP were sprayed in 3 lines on the detection strip of a lateral flow device. Goat anti-mouse IgG was lined (top) onto the detection strip as a positive control for the presence of Ab. Five mAbs to GP2 (1-5) and two mAbs to LASV NP (6,7) were applied to the sample well. Colloidal gold labeled anti-mouse Ab was present in the reagent reservoir. 4/5 GP2 mAbs and both GP2 mAbs bound specifically.

Lassa fever lateral flow diagnostic assays. Efforts have begun to develop Lassa fever lateral flow assays (similar to pregnancy tests) to provide a point-of-care diagnostic that can be useful in many situations, particularly deliberate release scenarios where rapid diagnosis is critical and in West African villages without access to ELISA equipment. Recombinant LASV proteins (bacterial GP1, GP2 and NP) were sprayed individually onto strips in a later flow format and several of the newly developed LASV-specific mAbs (tagged with colloidal gold) were applied to test wells of the lateral flow devices (later flow diagnostics are described more fully in the **Experimental Plan**). Several of the mAbs reacted to their specific LASV Ag in this format, indicating that they are appropriate candidates for further development in the proposed Lassa fever Ag-capture lateral flow (Fig. 8). These results also indicate that the recombinant LASV proteins we have produced are appropriate antigens for Ab-capture lateral flow assays.

RESEARCH DESIGN AND METHODS

Immunoassay development is an iterative process that involves testing a variety of assay configurations for optimal performance with diverse sets of patient derived samples. Commercial development further requires that components of the assay be produced in bulk scale with strict QA/QC standards. FDA guidance for commercial ELISA requires design, manufacturing and test performance standards that must be achieved prior to marketing. In this application we propose to perform essential steps in the preclinical development of commercial Lassa fever recombinant diagnostics. Expected results, potential pitfalls and alternative approaches for the plan presented here are provided in the RFA-required section **Milestones and Timelines**.

HYPOTHESIS: Commercial diagnostic assays for Lassa fever can be developed using recombinant LASV antigens and monoclonal antibodies that will have utility in bioterrorism scenarios and in endemic areas of West Africa.

The overall goal of our proposed study is to achieve critical milestones in preclinical development of Lassa fever recombinant diagnostics.

Table 3. Performance Panel for evaluation of recombinant Lassa fever antigen- and antibody-capture ELISA and lateral flow assays.

Sample	Number
Lassa fever early acute infection – Sierra Leone	18
Lassa fever late acute infection – Sierra Leone	18
Lassa fever convelescent – Sierra Leone	18
Non –Lassa febrile illness – Sierra Leone	18
Healthy controls – Sierra Leone	18
Lassa fever early acute infection – Other West Africa*	12
Lassa fever late acute infection – Other West Africa*	12
Lassa fever convelescent – Other West Africa*	12
Non –Lassa febrile illness – Other West Africa*	12
Healthy control - Other West Africa*	12
Non –Lassa febrile illness – United States	15
Healthy Controls - United States	15
Autoimmune patients - United States	12
Total	**192**

*Liberia, Guinea, Nigeria

MILESTONE 1: Development of commercial recombinant Lassa fever ELISA.

1A. Optimize commercial grade recombinant LASV antigen-capture and IgM- and IgG-capture ELISA using a Performance Panel of well-characterized sera.

Rational. Our Preliminary Results strongly suggest that Lassa fever immunodiagnostic assays using recombinant LASV proteins can be developed with superior sensitivity and specificity compared to assays based on LASV grown under BSL-4 conditions. The prototype Lassa fever recombinant ELISA will now be optimized for use as commercial assays according to strict design, manufacturing and test performance standards, and tested against diverse sets of serum samples to determine preclinical feasibility for biodefense and public health use.

Development of a Performance Panel for evaluation of recombinant Lassa Fever ELISA. In preliminary studies we developed prototype Lassa fever recombinant Ag- IgM- and IgG-capture ELISA. In field-testing these prototype assays out-performed Lassa fever ELISA based on BSL-4 grown LASV, as well as several versions of PCR-based LASV detection assays (Preliminary Results). To accomplish optimization of the Lassa fever recombinant ELISA we will assemble a Performance Panel consisting of sera from a well-characterized set of Lassa fever and non-Lassa fever patients (Table 3). The Performance Panel will include serum samples obtained across the area of West Africa that is known to be endemic for Lassa fever. Field collection of human serum samples from West Africa for the Performance Panel and other samples necessary for broader preclinical feasibility testing protocols is described in Milestone 5.

LASV demonstrates a high level of genetic diversity (Bowen et al., 2000), and it is impossible to predetermine which strain may be selected for a bioterrorism attack. Therefore, we propose to test the Lassa fever recombinant ELISA with samples obtained across the geographic range of LASV, including samples from Sierra Leone, Guinea, Liberia and Nigeria. Serum samples from patients early in the course of acute LASV infection (Ag positive, IgM negative), samples from later in acute infection (Ag negative, IgM positive) as well as convalescent patients (Ag and IgM negative, IgG positive) will be included in the Performance Panel. The major utility of Lassa fever diagnostics will be to distinguish Lassa fever patients from those with other febrile illness, both in bioterrorism and public health scenarios. Therefore, we will also include African patients with non-Lassa fever febrile illnesses (malaria, typhoid fever, other infections) in the Performance Panel. The Performance Panel will also include sera from a cohort of healthy West Africans, with a few expected to have IgG specific for LASV. Because the primary market for the Lassa fever diagnostics will be for biodefense, non-Lassa fever serum samples from persons living in the United States will also be included in the Performance Panel. Another subset of included sera will be from patients that are convalescent from acute *E. coli* and other gram-negative bacterial infections. Autoimmune patients with elevated levels of immunoglobulins and autoantibodies will also be included in the Performance Panels as sera from such patients can confound immunoassays.

Table 4. Comparison of Traditional and Recombinant IgM-capture ELISA

Traditional IgM capture ELISA			Recombinant IgM capture ELISA[1]		
Step	Component	Time	Step	Component	Time
1[1]	Anti-human IgM	1 hour	-	Recombinant LASV NP, GP1 and 2	precoat
2[1]	Patient serum	1 hour	1[2]	Patient serum	30 min.
3[1]	LASV- or mock-infected cell lysates	1 hour	-		
4[1]	Rabbit anti-LASV	1 hour	-		
5[1]	HRP-conj. anti-rabbit IgG	1 hour	2[2]	HRP-conj. anti-human IgM[3]	30 min.
6	Tetramethylbenzidine (TMB); 2 N H2SO4 (stop); read absorbance at 450 nm	5-15 minutes	3	Tetramethylbenzidine (TMB); 2 N H2SO4 (stop); read absorbance at 450 nm	5-15 minutes

[1]plates are washed 5X after this step in PBS-Tween 20
[2]plates are washed 4X after this step in PBS-Tween 20
[3]HRP-conj. anti-human IgG is used in the IgG-capture ELISA.

Additional characteristics of sera to be included in the Performance Panel are the availability of at least 5 ml of sera (alloquoted into 100 µl alloquots) and complete and well-documented clinical histories of the patients from which the sera were derived. Because it is essential that the ELISA developed have the ability to detect various strains of LASV, we will obtain a detailed sequence characterization of the viruses isolated from the panel with complete sequences of the GP and NP genes from each virus (See Milestone 5). Serum samples from patients infected with LASV that display maximum sequence diversity in both GP and NP will be included in the Performance Panel. The use of a well-characterized Performance Panel will allow various iterations of the Lassa fever assays to be directly compared and optimized, while controlling for a range of serum sample variation. The number of samples (192) we have selected is large for a Performance Panel, and it will be possible to evaluate/optimize several aspects of Lassa fever immunodiagnostic performance with a smaller numbers of selected samples. Subsets of the Performance Panel (48 and 96) samples will be selected and utilized where appropriate in certain aspects of the Lassa fever immunoassay development.

However, all 192 samples can be tested in two 96 well ELISA plates, and will be used against the final prototypes to determine standard protocols/configurations for the various versions of the Lassa Fever ELISA.

IgM- and IgG-capture ELISA. For prototype IgM- and IgG-capture ELISA, microplates will be coated with purified recombinant antigens produced in *E. coli*. The BSL-4-derived IgM-capture ELISA tests use a different protocol than we propose in the current studies (Table 4). Despite the significantly reduced complexity and shorter run time, the prototype recombinant assays were more sensitive than the corresponding BSL-4 assays (Table 2, Fig. 7). The prototype recombinant IgM and IgG ELISA will be further optimized and validated against the serological Performance Panel (Table 3). Systematic rounds of checkerboard titrations will be used to determine the optimum dilutions/source of recombinant LASV proteins (GP1, GP2 and NP), buffer compositions, and detection reagents to be used in the recombinant ELISA. If the bacterially produced LASV antigens fail to provide adequate sensitivity or specificity against the sera in the Performance Panel or the broader range of sera used in the preclinical feasibility studies described under Milestone 5, we will determine if the inclusion of LASV glycoproteins (GP1, GP2, GPC or NP) produced in mammalian cells improves performance of the ELISA. It may prove important to add recombinant proteins from isolates of LASV that display genetic diversity from the prototype (Josiah) strain.

Table 5. Comparison of BSL-4 and Recombinant Ag-capture ELISA

BSL-4 Ag capture ELISA			Recombinant Ag capture ELISA		
Step	Component	Time	Step	Component	Time
-	mAb to BSL-4 LASV	Pre-coat	-	mAb to recombinant LASV NP, GP1 and 2	Pre-coat
1[1]	Patient serum (1:2 1:5 1:10)	1 hour	1[2]	Patient serum (1:10 to 1:100)	30 min.
2[1]	Rabbit anti-BSL-4 LASV	1 hour	2[2]	HRP-conj. goat anti-LASV NP, GP1 and 2	30 min.
3[1]	HRP-conj. anti-rabbit IgG	1 hour	3	Tetramethylbenzidine (TMB); 2 N H2SO4 (stop); read absorbance at 450 nm	5-15 minutes
4	Tetramethylbenzidine (TMB); 2 N H2SO4 (stop); read absorbance at 450 nm	5-15 minutes			

[1]plates are washed 5X after this step in PBS-Tween 20
[2]plates are washed 4X after this step in PBS-Tween 20

Antigen-capture ELISA. The current Ag-capture Lassa fever ELISA uses microplates coated with five LASV-specific mAb produced at USAMRIID after immunization of mice with BSL-4 grown LASV (Table 5). The most potent of these mAbs is directed to LASV NP. To support commercial development of the recombinant Lassa fever Ag-capture ELISA, we have derived approximately 130 mAbs against LASV GP1, GP2 and NP (bacterial or mammalian cell produced; see Preliminary Results), which will be substituted for the USAMRIID mAbs in the commercial version of the LASV Ag-capture ELISA. As many as 10 murine mAb against LASV glycoproteins and NP will be coated on the commercial version of the Lassa fever Ag-capture ELISA plates. The composition of this mAb mix, as well as the amount of each mAb used will be determined in an iterative manner, guided in part by further characterization of antibody specificity as described under Milestone 4, as well as empirically by a series of checkerboard titrations against the Performance Panel.

The first step of both the BSL-4 and commercial Ag-capture ELISA will be addition of patient sera to plates precoated with LASV-specific mAbs (Table 5). The next step is the addition of the serum sample. The former

assay uses the patient sera at a 1:10 concentration. However, we have seen strong performance of the prototype commercial Ag-capture ELISA with a 1:100 dilution of patient sera. The amount of sera to be used in the commercial assay will be determined using the Performance Panel. After incubation and washing, a rabbit anti BSL-4 grown LASV serum is added, horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG antibody are added and the plates are again incubated. Finally, the plates will be washed, substrate will be added, and the plates incubated for 60 min and read. The second step of the BSL-4 Lassa fever Ag-capture ELISA uses a rabbit antiserum raised against BSL-4 grown LASV to detect antigen captured from the sera by the mAbs. In a third step, the BSL-4 assay uses a HRP-conjugated goat anti-rabbit antibody for detection of the Ag-rabbit anti-LASV complexes. The rabbit sera used in the former assay was raised to BSL-4 LASV grown in primate cells (Vero) and produces a high background in the assay, due to the production of anti-primate antibodies that react to human serum proteins (Preliminary Results). In contrast, the rabbit sera in the prototype Ag-capture assays was raised to recombinant LASV proteins, and appears to produce a high level of sensitivity and specificity. We have also raised high titer goat antibodies to recombinant LASV proteins. The Lassa fever Ag-capture assay can be further simplified by direct HRP conjugation of the goat antibodies to the recombinant LASV GP and NP. Further commercial assay optimization will involve an extensive adjustment of the mAb and goat anti-LASV serum dilutions, and conjugate reagents, substrates and buffers to achieve optimum sensitivity and specificity against the diverse serum samples in the Performance Panel. It may also be necessary to include mAbs raised to divergent LASV proteins, such as those derived from Nigerian isolates, as capture reagents.

MILESTONE 2: Development of Lassa fever lateral flow point-of-care diagnostics
2A. Develop LASV antigen-, IgM- and IgG-capture lateral flow assays.

Rational. A mandate of the current RFA (RFA-AI-08-001) is that the diagnostic assays developed be *"effective, rapid, highly sensitive, specific, easy to use, adaptable, and cost-effective."* While the ELISA described under Milestone 1 certainly fit these criteria, other Lassa fever diagnostic formats can have important applications in bioterrorism and public health scenarios. As discussed above (**Significance**) a point-of-care diagnostic can be particularly important for health facilities in developing countries where Lassa fever is endemic and where the number of patients that fit the differential diagnosis of this disease can be considerable. Unfortunately, these facilities often lack laboratory facilities to perform the required laboratory tests to confirm the diagnosis. The possibility of having laboratory tests done quickly and results available immediately is essential for Lassa fever, a severe and life-threatening disease which requires prompt medical intervention with ribavirin. The assay can also find use as a point-of-care test in bioterrorism scenarios.

Lateral flow assays for detection of LASV antigens, anti-LASV IgM or IgG. Rapid and effective lateral flow assays for serodetection of LASV antigens, and LASV-specific IgM or IgG will be developed using recombinant reagents. Lateral flow assays consist of a porous nitrocellulose detection strip flanked at one end by a reagent pad and at the other end by an absorption pad. A sample application pad flanks the reagent pad (Fig. 8). The composite strip is contained in a plastic assay device with a round sample well positioned above the sample application pad and a test result window positioned above the detection zone of the strip. The detection zone contains of at least 2 distinct lines, a test line and a control line, obtained by spraying antigens or antibodies onto the nitrocellulose strip using a device which is similar to an ink jet printer. Detection reagents in the reagent pad are labeled with colloidal gold or colored latex beads. The control line should stain in all cases. If the test line does not stain, the test is negative. The test is scored positive when the test line stains. Some variants of the assay, as in pregnancy tests, apply the control and test lines crossed at right angles, so that the control line (-) denotes a valid negative test, while a plus (+) denotes a valid positive test. Positive results may be subjectively rated 1+ when staining is weak, 2+ when staining is moderately strong, 3+ when staining is strong, and 4+ when staining is very strong. It is desirable for the sealed assay devices to be stable when stored at room temperature without loss of activity, and for the stain of developed tests to be stable after drying.

Lassa fever IgM- or IgG-capture lateral flow assays. For the proposed Lassa fever Ab-capture lateral flow

assay, recombinant GP1, GP2 and NP will immobilized on the cellulose acetate matrix. Affinity-purified antibodies against human IgM chemically linked to either colored latex beads or 40 nm colloidal gold particles will be contained in a reagent pad for detection. Tests are performed by the addition of 5 to 10 µl of serum to the sample pad of the assay device followed by the addition of 100 to 150 µl of sterile running buffer consisting of phosphate-buffered saline, pH 7.6, containing 1.67% bovine serum albumin and 3% Tween 20. Test results are read after 10 min by visual inspection for staining of the antigen and control lines. Serum added to the sample pad will flow into the trapping antibody to form a visible band as well as a second, control band further along the acetate strip. The control band consists of human IgM immobilized on the matrix. The IgG capture is configured similarly, except that affinity-purified antibodies against human IgG antibodies are included in the reagent pad, and the control line is human IgG

Lassa fever antigen-capture lateral flow assay. For the proposed Lassa fever Ag-capture lateral flow assay, mAbs specific for GP1, GP2 and NP will immobilized on the cellulose acetate matrix. A goat antibody to LASV GP1, GP2 and NP chemically linked to either colored latex beads or 40 nm colloidal gold particles will be contained in a reagent pad for detection. Tests are performed by the addition of 5 to 10 µl of serum to the sample pad of the assay device followed by the addition of 100 to 150 µl of sterile running buffer. Test results are read after 10 min by visual inspection for staining of the antigen and control lines. Serum added to the sample pad will flow into the trapping antibody to form a visible band as well as a second, control band further along the acetate strip. The control band consists of recombinant LASV proteins immobilized on the matrix.

2B. Optimize the lateral flow assays as point-of-care diagnostics using a Performance Panel of well-characterized sera.

As detailed above for the Lassa fever recombinant ELISA, lateral flow assays will be optimized for sensitivity and specificity using the Performance Panel (Table 3). Optimization will involve an extensive adjustment of the anti LASV mAb and goat anti-LASV serum dilutions, conjugate reagents, substrates and buffers to achieve optimum sensitivity and specificity against the diverse serum samples in the Performance Panel. Results with Lassa fever recombinant ELISA will provide guidance as to whether it will be necessary to include mAbs raised to divergent LASV proteins, such as those derived from Nigerian isolates, as capture reagents. The use of the Performance Panel will allow preliminary evaluation of the relative sensitivities and specificities of the Lassa fever ELISA and lateral flow assays.

MILESTONE 3: Manufacturing of ELISA and lateral flow assays under GMP.
3A. Convert to manufacturing of ELISA and lateral flow assays under Good Manufacturing Practices (GMP) with Quality Assurance (QA)/Quality Control (QC) to provide quantities of commercial grade diagnostic kits sufficient for preclinical evaluation of design control parameters to achieve benchmarks required for FDA approval.

Rational: A total of six diagnostic assays based on recombinant proteins are to be developed. LASV Ag-, IgG-, and IgM-capture assays will be developed in both the quantitative microplate ELISA and qualitative lateral flow assay formats to meet the needs of customers in both first-world and third-world countries. For each assay, pilot lots of kits will be manufactured by trained personnel using controlled documents. These pilot lots will be used to establish compliance with appropriate specifications (e.g., kit stability, temperature dependence, precision, limit of detection, interference and cross reactivity, recovery, and linearity). Based on a limited Performance Panel (Table 3) and clinical feasibility (preclinical) trials (Table 6), sensitivity and specificity will be determined. Specifications and acceptance criteria are discussed in detail for microplate ELISAs and lateral flow diagnostics below.

Stability. Real-time stability testing will be performed on all microplate and lateral flow kits. For each product, the first three lots manufactured for commercial sale shall be tested for Stress by Opening Monthly (SOM) and Specificity/Sensitivity (S/S) as per Corgenix Standard Operating Procedures (SOPs). In SOM testing, an

individual kit is removed from refrigerator storage and brought to room temperature to confirm that the cycling of temperatures (as would be experienced during normal use by the customer) will not affect assay performance during the anticipated life of the kit. In the quarterly S/S testing procedure, a series of up to 40 positive and negative patient samples and samples spiked with recombinant viral proteins are tested with each kit type to ensure the product continues to meet clinical specificity/sensitivity specifications throughout the shelf life. The kits will be considered to be stable if positive samples remain positive and negative samples remain negative for 1 year from the date of manufacture.

Temperature. As the temperature may vary considerably in various laboratories, particularly those laboratories in Africa, kits from each of three pilot lots will be tested at temperatures ranging from 18 to 35°C. Using negative and positive patient samples and/or samples spiked with recombinant viral proteins, results will be evaluated across the temperature range for all six assays. The tests will be considered to be successful if similar overall assay results are produced over the range of temperatures.

Precision. In the microtiter plate assays, both intra-assay precision (repeatability precision or within a plate) and inter-assay precision (within lab precision or between plates of a lot) will be tested in the assays in the microtiter plate format according to Clinical and Laboratory Standards Institute (CLSI) Document EP5-A2 "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline." Precision is not an issue in the qualitative lateral flow format. Multiple operators in multiple laboratories will test positive and negative samples with replicates in the three pilot lots for at least 20 days. The repeatability measure, based on the "within" variance component (i.e., the variability or a measurement within a plate), and the within lab measurement, based on the total variance component (within and between plates within a lot) and the lot-to-lot variability, will be calculated from these data. The goal for all precision measurements is $\leq$ 20%. In the lateral flow assays, reproducibility will be established according to CLSI Document EP12-A "User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline." In addition to the general reproducibility studies using the kit controls, the reproducibility of the assays at analyte concentrations near the cutoff will also be ascertained.

Limit of Detection (LOD)/Limit of Quantitation (LOQ). Determination of the limits of detection for the microtiter plate-based assays will be performed as stated in the CLSI Document EP17-A "Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline." This method is used to determine the smallest amount that can be reliably detected to establish the presence or absence of an analyte (LOD), and also the lowest concentration of analyte that can be reliably quantitated (LOQ). Measurements from pooled low-level samples (either patient samples or control samples spiked with recombinant proteins) and negative (Lassa-free) samples tested on three different pilot lots will be used.

Interference and Cross Reactivity. Interference testing will be performed as stated in the CLSI Document #EP7 "Interference Testing in Clinical Chemistry; Approved Guideline." Various materials likely to be found in blood and thought to have a potential to cause a false-positive or false-negative result will be tested at different concentrations to determine whether they interfere with LASV detection on both the microplate and lateral flow assays. Analysis will occur in at least two separate pilot lots using both negative and positive samples (and samples spiked with recombinant proteins). The assay will pass if none of the interfering substances cause greater than 20% difference from the results of samples without interferents in the microtiter plate assays and if a false-positive or false-negative result is not obtained in the lateral flow format. Additionally, assay kits will be tested for cross reactivity with samples positive for other viral illnesses resulting in fever and other Lassa fever-like symptoms, such as malaria and typhoid fever. The goal of these tests is that none of these agents will cross react with the Lassa fever assays to produce a false-positive result.

Linearity/Recovery. Linearity studies will be performed following the CLSI guideline document EP6 "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline" on the pilot lots. These tests determine a range of sample concentrations that result in accurate test values. Briefly, patient samples or normal samples spiked with recombinant proteins will be used, both

with a high level of reactivity and with a low level. Samples are diluted together in fixed ratios and the degree of linearity is determined for each assay. Successful linearity testing will result in a linear dilution range of patient samples from 1:100 to 1:2000.

Sensitivity/Specificity. The relative sensitivities and specificities of the antigen- and antibody assays in the microtiter and lateral flow assays will be determined as described in CLSI Document I/LA21-A "Clinical Evaluation of Immunoassays; Approved Guideline." CLSI Document EP12-A "User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline" will be the basis for analysis of the lateral flow assay results. Acceptable ranges for assay sensitivities and specificities are discussed under the RFP-required section **Milestones and Timelines.**

Regulatory requirements. The plan for compliance with regulatory requirements, including the use of GMP facilities, document controls, and interactions with other members of the development team is discussed under the RFP-required **Product Development Plan**.

MILESTONE 4: Process development for production of recombinant LASV proteins and LASV-specific mAbs.

Rationale: We have expressed recombinant LASV proteins in both bacterial and eukaryotic cell systems at a scale sufficient for the development of prototype Lassa fever ELISA, and for production of mAbs. We will continue to use Rosetta strains of *E. coli* to express recombinant LASV proteins as MBP fusions for Lassa fever ELISA and lateral flow assay development as these proteins performed well in prototype assays. However, increased LASV protein production, and more efficient methods than production as MBP fusions, is required for commercial production. To this end it is imperative that the expression level and final yields of purified, full length, correctly folded, immunologically reactive recombinant LASV proteins per unit of cell mass are maximized. In parallel with commercial development of our ELISA and lateral flow assay, we will optimize methods for commercial production of LASV proteins. Proteins produced by alternative methods will be tested in the optimized assays to ensure that they perform as well or better than current proteins. We anticipate that no single platform will be optimal for expression of each LASV protein (NP, GP1 and GP2) at commercial scale. Methods to be developed by Autoimmune and Vybion, include:

- Use of self-cleaving (intein) domains in *E. coli* Rosetta 2(DE3)
- Codon-optimization for maximum expression in BL21(DE3) or other *E. coli* strains.
- Purification of proteins from inclusion bodies, followed by refolding.
- Expression of LASV recombinant proteins in *Pichia Pastoris* or mammalian cells.

Development of Lassa fever Ag-capture recombinant ELISA and lateral flow assays requires production of a robust set of broadly-reactive mAbs produced at commercial scale.

MILESTONE 4A Optimize scale up/purification of recombinant LASV proteins, GP1, GP2, and NP.

Optimization of recombinant LASV NP, GP1 and GP2 production in E. coli Rosetta 2(DE3). Current fermentation of LASV NP, GP1 And GP2 as MBP fusions, and subsequent purification on amylose resin, cleavage with Factor Xa, and size-exclusion chromatography (SEC) is outlined in Preliminary Results and in Branco et al., 2008. Greater than 85% of LASV NP and 90% of LASV GP1 produced by current methods is full-length protein. A minor fraction of NP is a truncated 46 KDa fragment that appears to arise as a prematurely terminated translation product. Minor contaminants of 33, 16 and 13 KDa in size account for less than 10% of other protein content in most GP1 preparations. Western blot analysis revealed that currently ~ 20% of LASV GP2 generated in Rosetta cells is full-length, and the remaining 80% is identified as a truncated 13 KDa fragment of the protein. SDS-PAGE also revealed an additional ~16KDa contaminant in the GP2 preparation that is identified as a cleaved fragment of MBP. The use of proteases, such as factor Xa that uncouple a fusion protein partner from the recombinant viral protein of interest are not optimal for full

scale commercial production. There are issues of cleavage sequence specificity, removal of protease after cleavage, lot-to-lot variability in terms of purity, activity and specificity, contaminants, potential introduction of endotoxin or adventitious agents (Factor Xa is purified from bovine serum), and high cost per unit of enzyme. Technologies that employ self-cleaving protein domains fused to the protein of interest are ideal candidates to consider when developing and scaling up purification methods for generation of large quantities of proteins for preclinical studies (Banki and Wood, 2005; Wu et al., 2006). The LASV NP, GP1 and GP2 genes have been cloned in the pMIT vector as C-terminal fusions, which contain the MBP gene sequence (M) fused to the *Mycobacterium tuberculosis* (Mtu) *recA* intein (I), followed by the LASV sequence. Optimization studies are now required to establish optimal fermentation medium, temperature, induction conditions, purification resins and parameters, and cleavage conditions that yield the highest levels of purified LASV NP, GP1 and GP2 protein. *E. coli* Rosetta 2(DE3):pMIT-NP, -GP1 or -GP2 will be grown in fermentors to high densities (A_{600}>75) in fully chemically defined (CD) media to significantly increase final cell mass per liter of culture. CD media will be tailored to increase yields of full-length recombinant protein. Intein-mediated self cleavage will be optimized to yield the highest possible amount of LASV NP, GP1 or GP2 per gram of cell paste, at the lowest possible cost per unit of protein, with final purities greater than 90%.

Codon-optimization for maximum expression in other E. coli strains. A subset of the codons for Arg, Ile, Gly, Leu, and Pro are rarely used in *E. coli* genes. When the mRNA of heterologous target genes, such as those of LASV, a eukaryotic virus, are over-expressed in *E. coli*, differences in codon usage can impede translation due to the demand for one or more of these tRNAs. Insufficient tRNA pools can lead to translational stalling, premature translation termination (e.g. for LASV GP2), and translation frameshifting or amino acid misincorporation. The Rosetta strains of *E. coli* increase the amount of various rare-for-*E. coli* tRNAs via incorporation of a multiple copy plasmid. While this allows expression and yields of proteins, such as LASV NP, GP1 and GP2, whose genes contain rare codons to be improved, the Rosetta strains typically cannot be grown to as high densities as other *E. coli* strains and maximal yield of proteins are somewhat low by commercial standards. To increase production yields, the LASV genes will be codon-optimized for maximum expression in an *E. coli* strain such as BL21(DE3), which is amenable to scale-up production. The genes will be expressed from the inducible T7 RNA polymerase promoter in plasmid pET24a (Novagen Inc) to ensure that expression does not accumulate during build-up of biomass, but rather, following peak level of biomass to avoid potential toxic effects. Optimization of culture growth will be carried out in 10L scale batch fermentation or fed-batch fermentation where parameters including media composition, growth and induction temperature, and time and length of induction can be monitored and optimized.

Purification of proteins from inclusion bodies. Purification of proteins from insoluble inclusion body fractions generated during *E. coli* fermentation by high throughput refolding studies can also yield high levels of recombinant proteins. Previous studies indicated that a majority fraction of the LASV NP, GP1, and GP2 expressed from pMAL-based vectors in *E. coli* Rosetta cells is insoluble material. Culture conditions can be adjusted to significantly increase yield through overexpression of proteins intracellularly in denatured form as inclusion bodies. Typically, the inclusion bodies are solubilized in guanidine followed by dilution of the denaturant into a buffer with specific excipients to maintain solubility and subsequent refolding of the protein through buffer exchange into a minimal buffer. Vybion routinely refolds functional recombinant proteins produced in *E. coli* inclusion bodies, with the largest being a pentamer of identical 27 kDa monomers (serum amyloid P component).

Expression of LASV recombinant proteins in Pichia Pastoris. An alternative to production in *E. coli* would be expression of the LASV genes in the yeast *Pichia Pastoris*. Vybion has considerable experience with the *Pichia* expression system having produced a line of 15 products including 9 viral proteins derived from the HBV, HSV, and HIV genomes. High yields of expressed protein are attainable because of the high biomass that can be reached in fermentors and the strong methanol-inducible promoters that drive high-level expression from the vectors pPIC3K and pPIC9K. Additionally, expressed viral genes become integrated in multiple copies in the *Pichia* genome, thus, leading to increased expression. Typical biomass yields obtained by Vybion average around 350 g/L of which the expressed protein can represent up to 2 g/L. To aid in purification, the LASV proteins can be secreted into the medium via the γ-factor secretory signal present on

pPIC9K. Proteins are expressed extracellularly at 0.5-1 g/L, however, the lower yield is offset by the increased purity of the initial raw material prior to downstream chromatographic processing. A potential downside to expressing LASV proteins extracellularly is that they become glycosylated (although not hyperglycosylated like proteins produced in *Saccharomyces cerevisiae*). Potential glycosylation sites within the LASV proteins can be identified prior to expression to assess whether glycosylation would occur and glycosylation (or lack of glycosylation) can be verified by the appearance of high MW product via PAGE-gel analysis.

Expression of LASV recombinant proteins in mammalian cells. Recent efforts have resulted in the successful expression of LASV GP1 and GP2 as soluble, secreted standalone proteins in mammalian cells (Figs. 3, 4). Likewise, LASV NP has been expressed and purified from mammalian cells (Fig. 4). Recent advances in transient mammalian cell expression technologies permit the scale-up and easy purification of multi-milligram quantities of high quality and native or quasi-native proteins in a few days. Thus, the infrastructure has been generated for the optimization of recombinant LASV protein generation using cost effective and expedited approaches across a multitude of expression platforms.

LASV sGP1-FLAG, sGP2-FLAG, NP-HIS: Culture supernatants from HEK-293T/17 or 293-F cells transiently transfected with construct pcDNA3.1+_intA:sGP1-FLAG are loaded onto an Anti-FLAG M2 agarose column, followed by extensive washing. sGP1-FLAG is eluted with FLAG peptide or low pH Glycine buffer, and fractions are analyzed by SDS-PAGE and Western blot, and sGP1-FLAG- containing fractions are pooled. The sGP1-FLAG eluate pool is concentrated, dialyzed against PBS, sterile filtered, vialed and stored at -20°C. Greater than 98% of LASV GP1-FLAG produced by this method is full-length protein (see Figure 7B). A minor high molecular component that is recognized by anti-GP1 specific mAbs and that corresponds to twice the fully glycosylated molecular weight of sGP1, or ~ 85 KDa, can be readily detected in all preparations by Western Blot analysis. This dimer aggregate appears to be irreversible, as it cannot be disrupted under reducing SDS-PAGE conditions. Future batches of sGP1-FLAG will be purified from transiently transfected CHO-S or stably transfected CHO DG44 CD-SFM culture supernatants. The best producing stable cell lines will be grown in shaker flasks, spinners, or bioreactors under controlled conditions to yield the maximum attainable levels of recombinant sGP1-FLAG per liter of spent medium. The addition of mild solubilizing agents, such as Pluronic F-69 and other vendor proprietary anti-clumping reagents, will be investigated as a means to prevent aggregation of sGP1 in the cell culture medium, thereby decreasing the levels of dimer. A similar approach will be employed for the purification of sGP2-FLAG from the supernatants of cells transfected with pcDNA3.1+_intA:GPCDTM-FLAG.

LASV NP-HIS: Protein extracts of HEK-293T/17, 293-F, or CHO-S cells transiently transfected with construct pcDNA3.1+_intA:NP-HIS are prepared by solubilizing cell pellets in a Tris-EDTA/ NaCl/ Deoxycholic acid/ IgePal CA-630/ SDS buffer containing a protease inhibitor cocktail. The insoluble fraction is pelleted by centrifugation and supernatants are loaded onto a metal ion resin column, followed by extensive washing. Protein is eluted with Imidazole buffer, and fractions are analyzed by SDS-PAGE and Western blot, and NP-HIS- containing fractions are pooled. The NP-HIS eluate pool is concentrated, dialyzed against PBS, sterile filtered, vialed and stored at -20°C. Greater than 98% of LASV NP-HIS produced by this method is full-length protein (see Fig. 7A).

Purification of recombinant LASV proteins. Downstream processes for purification of the LASV proteins for use in validation and commercial development studies will be developed. Such processes including chromatographic purification, analytical analysis, assay development and stability studies and subsequent scale-up. Chromatographic purification would include the development of a scaleable cleavage/purification process to separate the fusion partner MBP from the LASV proteins. Separation of the cleavage products by size-exclusion chromatography is, however, limited by a loss of resolution upon scale-up and additional chromatographic steps may be necessary. Other alternative discussed above express the LASV proteins without the MBP moiety and will require purification via conventional chromatography.

4B. Optimize scale up/purification of mAbs to recombinant LASV GP1, GP2, and NP.

Mouse immunization with recombinant LASV proteins and derivation of mAb. BALB/c mice were immunized with recombinant LASV GPC, GP1, GP2 and NP for the production of monoclonal antibodies. Hybridomas that secreted specific mAb were subjected to limiting dilution cell cloning (LDCC). Accession banks were prepared for each specific hybridoma and cryopreserved. Supernatants from hybridomas of interest were isotyped to facilitate assay development and selection of secondary reagents. Each hybridoma of interest will be subsequently adapted to growth in SFM for ease in subsequent scale-up and purification of mAb. Commonly, hybridomas adapt readily to SFM, and thus purification of mAb can be accomplished with well-established Protein A or G based procedures described below. The current SFM formulation of choice is PFHM (Protein-Free Hybridoma Medium) from Invitrogen, which has consistently accommodated adaptation of hybridomas in a short period of time from growth in T flasks to spinner flask cultures.

Monoclonal Ab will be evaluated for neutralizing activity in LASV plaque reduction and neutralization test (PRNT) assays. The identities of each mAb will be fine mapped at the molecular level by determining the nucleotide sequence of matched light and heavy chain gene sequences from each clonal hybridoma cell line. This approach will narrow the specificities to each LASV protein, and will reduce the number of mAbs to be analyzed further by iterative processes. Epitope mapping using overlapping peptides corresponding to LASV proteins is also currently being employed to provide information on epitopes of diagnostic important. Currently, four anti-LASV GP1, five anti-GP2, and five anti-NP mAbs are being evaluated as candidates the development of ELISA- and lateral flow-based diagnostic platforms. Each mAb was chosen based on its binding properties in ELISA and Western Blot formats, and isotype. Each mAb will be generated from a clonal hybridoma cell line that has been characterized by comparing its productivity, isotype, binding to antigen by ELISA and Western Blot relative to the uncloned parental isolate. Our preliminary results suggest that all clonal hybridoma cell lines will readily adapt to growth in SFM at high density in spinner flasks, and that productivity will range from ~15-200 mg/L

Protein A or G-based column chromatography methods will be used to purify the murine mAb from the hybridoma cell culture medium. The initial capture step of the antibody from the cell culture supernatant using Protein A or G chromatography typically yields pure mAb (over 90%). If required, a second chromatography step will be performed to remove any residual protein A or G and other minor contaminants. A final step will involve either gel filtration/SEC chromatography or dialysis (at the small scale) or ultrafiltration (at a large scale) to formulate the protein in the desired buffer with a specific ionic strength, pH, and osmolality. SDS-PAGE, size-exclusion or reverse-phase HPLC, UV spectrophotometry, ELISA, and Western Blot, where applicable, will be used to test in-process and final bulk material for protein purity and identity.

MILESTONE 4C Manufacturing with Quality Assurance (QA)/Quality Control (QC), to provide quantities sufficient for preclinical evaluation.

Manufacturing with Quality Assurance (QA)/Quality Control (QC). For each bacterial strain and mammalian cell line to be employed in the generation of recombinant LASV proteins for the proposed studies a working cell bank (WCB) will be generated and characterized. Characterized WCB will ensure input cell line consistency in each fermentation and cell culture scale up run. Characterization tests for mammalian WCB will be viability of thawed cryoculture, retention of expression cassette under selection conditions, and microbial contamination (sterility, Mycoplasma). Characterization tests for bacterial WCB will be viability of thawed cryoculture, viability in culture, retention of recombinant construct and of all selectable markers.

A reference standard will be generated for each mAb and LASV protein used in the studies outlined herein. The reference standard will be prepared under the same specifications as subsequent pre-clinical lots. The physiochemical testing of the reference standard will include total protein content, SEC-HPLC, Reversed Phase HPLC, reducing and non-reducing SDS-PAGE, MALDI-MS, and N-terminal sequencing. A new

reference standard will be prepared and characterized from material generated in each fermentation and cell culture manufacturing campaign.

Table 6. Anticipated number of samples available for evaluation of Lassa fever recombinant antigen- and antibody-capture ELISA and lateral flow assays (5 year totals).

Sample	Number
Lassa fever acute infection – Sierra Leone	600
Lassa fever acute infection – Guinea	150
Lassa fever acute infection – Liberia	150
Lassa fever acute infection – Nigeria	100
Lassa fever convalescent – Sierra Leone	800
Lassa fever convalescent – Guinea	200
Lassa fever convalescent – Liberia	200
Lassa fever convalescent – Nigeria	100
Non-Lassa febrile illness – Sierra Leone	800
Non-Lassa fbrile illness - Guinea	200
Non-Lassa febrile illness - Liberia	200
Non-Lassa febrile illness - Nigeria	100
Non-Lassa febrile illness – Argentina (Junin)	100
Non-Lassa febrile illness – Bolivia (Machupo)	10
Non-Lassa febrile illness – Venezuela (Guanarito)	10
Healthy control – Sierra Leone	1200
Healthy control - Other West Africa*	500
Healthy control - South Africa	200†
Non –Lassa febrile illness – United States	200
Healthy Control - United States	200
Autoimmune patients - United States	200
Total	**6220**

*Liberia, Guinea, Nigeria; †more available as needed.

Diluents for each LASV recombinant protein will be developed in accordance to experimentally determined purification parameters that sustain solubility and integrity in liquid formats. The formulation of recombinant proteins constitutes one of the last steps before the product is vialed in a fill/finish environment. Formulations are designed to maintain stability and potency of the protein product in solution or in lyophilized forms. Stability tests and specifications for individual diluents and protein formulations will be established. Analytical assay development and implementation will support the proposed lot release specifications, under established GLP guidelines. It is imperative that formulations perform buffering, cryo/lyo protectant, bulking, solubilizing, denaturation and shear protection functions in the final product. The stability of formulated proteins will be monitored at -80°C, -20°C, 2 – 8°C, 25 °C and 37 °C, and tested at 0, 3, 6, 9, 12, 18, 24, and 36 months post manufacture. Although the shelf life of individual LASV proteins has not been established, generation of reference standard on a yearly basis should suffice to meet the specifications set herein. Each lot of recombinant LASV protein will be subjected to acceptance criteria under the following release specifications: appearance (liquid), pH, protein concentration by A_{280} and BCA, purity by SDS-PAGE, SEC-HPLC and Western Blot, identity by ELISA and Western Blot, charge heterogeneity by IEF, relative binding by ELISA, residual DNA, and bacterial endotoxin by kinetic turbidimetric method.

MILESTONE 5: Field testing of commercial recombinant Lassa fever ELISA and lateral flow point-of-care diagnostics in West Africa.

Rationale. Development and validation of the commercial grade LASV assays will require access to a large number of diverse clinical specimens collected from patients with and without Lassa fever. A summary of the types and anticipated numbers of samples to be collected is provided in Table 6. For LASV-infected persons, serum from a broad spectrum of disease time-points, ranging from early infection through convalescence, will be necessary to delineate the temporal profile of the LASV antigen, IgM and IgG responses as detected by the various assays and to develop standard curves for each. Furthermore, samples from across the spectrum for Lassa fever in West Africa must be represented because four distinct geographic lineages of LASV have been shown to exist, with up to 23.8% nucleotide divergence in the NP gene (Bowen, Peters, and Nichol, 1996; Bowen, Peters, and Nichol, 1997; Bowen et al., 2000). Considerable intra-lineage

Fig. 9. Map of the Mano River Union countries (Sierra Leone, Guinea, and Liberia). The approximate known endemic area for Lassa fever in the region is shown by the dotted oval. Sites of the four laboratories included in the *Mano River Union Lassa Fever Network* are indicated by stars and consist of the Kenema Government Hospital Lassa Laboratory in Kenema, Sierra Leone; the International Center for Research on Tropical Infections in N'Zérékoré, Guinea; the Program on Hemorrhagic Fevers in Conakry, Guinea; and the Central Public Health Laboratory in Monrovia, Liberia.

sequence diversity has also been noted. The specificity of the assays for Lassa fever must be assessed by testing patients with arenavirus infections other than LASV, as well as patients with non-arenavirus febrile illnesses. Testing of an array of "normal" sera collected from healthy populations from both endemic and non-endemic areas for Lassa fever in Africa, as well as in the United States, will be required to assess signal-to-noise ratios in these different populations and set cut-offs for a positive result. Lastly, testing of samples from patients with autoimmune disorders is advisable to assess the possibility of non-specific cross-reaction that often occurs with sera from patients with these diseases.

Normally, collection of such a diverse panel of serum would be virtually impossible, especially when considering that the disease and virus in question is a VHF and Category A Select Agent. The geographic region with the highest incidence of Lassa fever consists of contiguous areas of Sierra Leone, Guinea, and Liberia (collectively know as the "Mano River Union" [MRU] countries) (Khan et al., 2008), one of the poorest regions of the world and, until recently, plagued by civil war (Fig. 9). However, through a unique new capacity building project in West Africa, the study team will have access to clinical samples from all three MRU countries, with a laboratory for real-time in-country diagnostics in Kenema, Sierra Leone. The reviewers are directed to the **RESOURCES** section of this application for a detailed description of the current clinical and laboratory capasity built by members of this team in West Africa. It is through this program that the majority of samples were obtained for our team's initial work developing and testing recombinent antigen-based ELISAs for Lassa fever. We will again profit from this valuable resource to execute the research program proposed here. Additional samples will come from Nigeria, Argentina, Bolivia, Venezuela, and elsewhere in Africa and the United States.

5A. Define and collect positive and negative sera for assay validation from diverse regions across the Lassa fever endemic range of West Africa and elsewhere.

Samples from the following groups will be collected for Lassa fever recombinant ELISA and lateral flow assay development:

Convalescent serum from patients with Lassa fever and healthy controls in West Africa. Follow-up visits with surviving patients will constitute the main source of convalescent sera for testing for IgM, which lasts for a few months after infection (Bausch et al., 2000), and IgG antibodies. However, the number of specimens from convalescent patients relative to the number with acute disease will diminish by approximately 50% due to death from the acute Lassa fever (approximately 25% of hospitalized patients) and loss-to-follow-up (another 25%, in our experience). However, the number of specimens from convalescent cases will be augmented by detecting IgM and/or IgG-positive persons through the outreach team's sampling with

informed consent of family members and other contacts during visits to the homes where cases originated, which is the standard public health practice. Mild or asymptomatic cases are often detected this way. Furthermore, Tulane is presently conducting a seroprevalence study for exposure to LASV in which over 2000 serum specimens (of a target 4000) from Kenema District have already been collected. As historically the seroprevalence of IgG antibody to LASV may approach 50% in some of the villages under study, this will constitute a major source of IgG positive sera. Furthermore, specimens that test negative for any exposure to LASV will be a major source of healthy control serum from West Africa. This number can be augmented, if need be, by specimens from numerous other population-based studies in which Tulane is presently engaged, as well as, if need be, from sample remainders from KGH. A similar approach will be taken in the other MRU-Lassa fever network (LFN) countries and Nigeria, with sample collection either through similar ongoing studies or collections specifically designed for this research proposal.

Sera from patients with non-Lassa febrile illnesses. Specimens from patients with non-Lassa febrile illnesses, as well as from healthy patients, will also be collected through the MRU-LFN. In our experience, slightly less than half of the patients tested at the KGH Lassa Laboratory are confirmed to have Lassa fever, although the disease was obviously suspected and the patient fulfilled the case definition. These LASV-negative patients form an obvious and appropriate source for the non-Lassa febrile illness sera. If necessary, the number of specimens can be relatively easily augmented through sampling with informed consent of patients with systemic febrile illnesses on the general medicine and pediatric wards.

Samples from patients with non-Lassa arenaviruses infections. As cross-reaction with other arenaviruses could compromise the specificity of the results of the ELISA for Lassa fever, we plan to test the developing and final commercial assay on sera from patients confirmed to have New World arenavirus infections. Toward that end Dr. Delia Enria, Director of the Instituto Nacional de Enfermedades Virales Humanas "Dr. J.I. Maiztegui" in Pergamino, Argentina, has graciously agreed to make available for testing a collection of approximately 100 serum specimens from patients with Argentine hemorrhagic fever (Junin virus infection) (Letter of Support attached). Dr. C.J. Peters of the University of Texas Medical Branch, Galveston, TX, has already make available a smaller number (~20) of serum samples in his possession from patients with Bolivian (Machupo virus) and Venezuelan (Guanarito virus) hemorrhagic fevers (Letter of Support attached).

Healthy patients-United States and South Africa. Serum samples from anonymous donors will be collected from 10 clinical sites in the United States, including Tulane Medical Center. A similar set of anonymous serum samples is available from South Africa and other areas of Africa outside of the Lassa fever endemic range (Robert Swanepoel, Letter of Support attached).

Patients with autoimmune disease. A collection of serum samples from anonymous patients, who provided informed consent to use their samples in future unspecified studies, with various autoimmune diseases seen at Tulane Medical Center and associated clinics, and elsewhere in the United States are available to investigators at Tulane and Autoimmune Technologies.

Institutional Review Board/Ethics Committee approval and informed consent. The research protocols and all consent/assent and data forms will be submitted to and approved by the Institutional Review Board (IRB) of Tulane and the IRBs or Ethics Committees of the implicated countries in Africa prior to implementation of the study. Informed consent will be obtained from all subjects prior to obtaining specimens for research. In some cases, execution of the study may entail testing anonymous sample remainders or serum specimens collected for other purposes. When appropriate, exemption from the requirement of IRB/Ethics Committee approval will be sought and obtained prior to testing the samples.

Patient identification and specimen collection. In the MRU countries, blood is normally drawn immediately upon clinical suspicion of Lassa fever (which is most often at the time of admission) and then at various intervals thereafter, depending on the clinical course. Samples are collected in 10-ml syringes and stored on wet ice or cold packs and delivered to the nearest MRU-LFN laboratory (usually not more than 3-4 hours away by road). Samples delivered to the KGH Lassa Laboratory (most often coming directly from the

adjacent Lassa ward) are tested immediately (see details below). Samples arriving to the laboratories in Guinea and Liberia are placed in the refrigerator and allowed to clot (centrifugation is discouraged to avoid potentially dangerous aerosols) and the serum and cells then separated by trained technicians wearing standard personal protective equipment (i.e. masks, gowns, and gloves). Samples are labeled and stored at –20°C until transport can be arranged to Kenema (usually <48 hrs), where they are then sent by Ministry of Health- or WHO-provided ground transport in specialized safety shippers on wet ice or cold packs.

Patient management and infection control. As Lassa fever is endemic in the MRU countries, the hospitals in the region habitually see patients with the disease and have standard protocols for infection control. The study proposed here does not consist of a clinical trial and, in most cases, will not seek to obtain specimens from acutely ill patients that would not ordinarily be acquired through the clinically indicated management. However, to insure optimal management of the patient and the safety of the medical staff, with the consent of the hospital administration, patient isolation procedures and facilities will be reviewed for each center and revised or up-graded as necessary. Training sessions in infection control will be conducted and an adequate supply of personal protective equipment and other necessary supplies for the optimal care of patients with Lassa fever, such as syringes and intravenous fluids, will be supplied to each hospital. Although ribavirin is supplied for KGH and other hospitals in catchment areas for Lassa fever by the respective countries' Ministries of Health, supplies are often inadequate. We will therefore supplement the supply, including the necessary intravenous fluids for delivery, to insure ribavirin's availability for all patients confirmed to have Lassa fever. All decisions regarding patient care will rest with the local treating physician.

5B. Test and compare the commercial Lassa fever recombinant ELISA and recombinant lateral flow point-of-care diagnostic assays with results from BSL-4 ELISA and PCR assays.

Diagnostic testing. Diagnostic testing for Lassa fever is performed within 24 hours of receipt of a specimen at the KGH Lassa Laboratory by ELISA antigen, IgM and IgG antibody using WHO-sanctioned conventional BSL-4 produced reagents (Bausch et al., 2000), and real-time PCR using three different primer sets (Demby et al., 1994; Drosten et al., 2002; Drosten et al., 2003). Results are conveyed immediately to the treating physician. Once this diagnostic testing is performed, the remaining sample (usually ~5 mls) will be available for research purposes. In addition to the testing using the BSL-4 ELISA and real-time PCR, each sample will be tested using the commercial recombinant LASV antigen capture, IgM and IgG capture ELISAs, as well as with the lateral flow rapid test for Lassa fever. Tests from convalescent patients will only be tested for LASV-specific IgM and IgG.

- A subset of ELISA Ag- and/or PCR-positive samples will be sent in specialized safety shippers approved by the International Air Transport Association and with all necessary permits to USAMRIID, followed by sequencing of the complete LASV GPC and NP genes. 300 µl of cell suspension will be added to 1 ml of Tripure reagent (Boehringer Mannheim, Indianapolis, Ind.) to inactivate virus and protect viral RNA from nuclease digestion. The samples will then be passed out of the BSL-4 laboratory through the Lysol immersion tank for RNA extraction and RT-PCR. Genetic sequence analysis by USAMRIID and Tulane scientists will be performed using established GP1, GP2 and NP primers that amplify the entire sequences encoding these LASV proteins.

Data analysis. The results from BSL-4 and recombinant-antigen ELISA for Lassa fever (Ag, IgM, and IgG), the lateral flow point-of-care assay, and PCR will be compared. Sensitivity, specificity, and positive and negative predictive values will be calculated using the BSL-4 ELISA as the gold standard, with a secondary analysis considering PCR (results from both individual primer pairs as well as from all primer sets taken together) as the gold standard to be compared with the recombinant ELISA antigen assay. The sensitivity and specificity of combinations of ELISA results, such as antigen and/or IgM positive to indicate acute LASV infection, will also be explored. As for other parts of this Experimental Plan, expected results, pitfalls and alternative approaches are discussed in the RFA-required **Milestones and Timelines** section that follows.

Milestones and Timeline

Our overall goal is to perform critical steps in the preclinical development of recombinant antigen Lassa fever immunodiagnostics during an aggressive 5-year work period. This project has 5 developmental Milestones (interim objectives) to be achieved during the course of the project. Each Milestone represents one or more Go-No Go decisions. Quantitative criteria by which milestone achievement will be assessed are described in the following narrative. We also discuss impediments that could require a revision in the work plan (or Milestones) with consideration of alternative approaches. A detailed schedule (timeline) for the anticipated attainment of each Milestone and the overall goal is provided graphically in Figure 10.

MILESTONE 1: Development of commercial Lassa fever ELISA.
1A. Optimize commercial grade LASV antigen-capture and IgM- and IgG-capture ELISA using a Performance Panel of well-characterized sera.
BSL-4 combined Ag/IgM ELISA has a sensitivity of 85-92% with specificity of 92-99% (Bausch et al., 2000, Table 2). Prototype Lassa fever recombinant ELISA outperformed the BSL-4 ELISA. We expect that the commercial recombinant ELISA developed herein will be more sensitive than the existing ELISA that use BSL-4 derived materials. Nevertheless, we will consider Milestone 1 to have been achieved if the commercial Ag-capture and IgM ELISA are equally as sensitive and specific as existing BSL-4 assays. This benchmark will provide a commercial basis and potential to make LASV ELISA widely available for biodefense, public health and research uses. If the pilot lots of the commercial LASV antigen-capture ELISA or recombinant antigen based IgM capture ELISA used in combination are substantially less sensitive or specific (<85% sensitivity, <92% specificity) than the existing ELISA produced using BSL-4 derived materials on the Performance Panel (Table 3), and we cannot increase the sensitivity to the levels achieved by BSL-4 ELISA in head-to-head competitions, then we will consider that Milestone 1 has not been achieved. This assessment will be completed by the end of grant year 2. If a No Go decision has been reached for Milestone 1, then no additional funding for the entire project will be accepted after year 2.

Expected results, potential pitfalls and alternative approaches. We expect that the approaches taken will lead to Lassa fever recombinant ELISA as sensitive and specific as currently available assays with the added advantages that they are scalable, commercializable and do not require BSL-4 facilities for production. Based on our preliminary results, it is likely that improvements in the sensitivity and specificity of the ELISA will be achieved.

MILESTONE 2: Development of Lassa fever recombinant lateral flow point-of-care diagnostics.
2A. Develop LASV recombinant antigen-, IgM- and IgG-capture lateral flow assays.
Our Preliminary Results suggest that the recombinant antigen-based reagents that we have developed are suitable for use in lateral flow formats. Specifically, we have shown that many of the mAbs recognize the LASV proteins in western blot or lateral flow assays (Fig. 8), and we anticipate that we will be able to configure robust Lassa fever lateral flow point-of-care diagnostic assays. If both LASV antigen-capture or recombinant antigen based IgM capture prototype lateral flow assays, cannot be configured with moderate (>80% sensitivity, >85% specificity) on the Performance Panel by the end of year 2, we will consider that Milestone 2A has reached a No Go decision. If this No Go decision is reached, no additional funding for development of lateral flow assays will be accepted after year 2, but we will renegotiate reduced levels of funding to pursue recombinant ELISA as the sole Lassa fever diagnostic platform.

2B. Optimize the recombinant lateral flow assays as point-of-care diagnostics using a Performance Panel of well-characterized sera. We do not expect that optimized commercial-grade lateral flow assays will be as sensitive as the recombinant ELISA, but we do expect to achieve minimal benchmarks of >85% sensitivity and >92% specificity. Some reduced sensitivity and specificity is acceptable for a point-of-care assay that will provide rapid diagnosis in deliberate release scenarios, or for public health uses in resource-poor areas of West Africa. If the pilot lots of the LASV antigen-capture or recombinant antigen based IgM capture lateral flow assays used in combination cannot be developed with >85% sensitivity and >92%

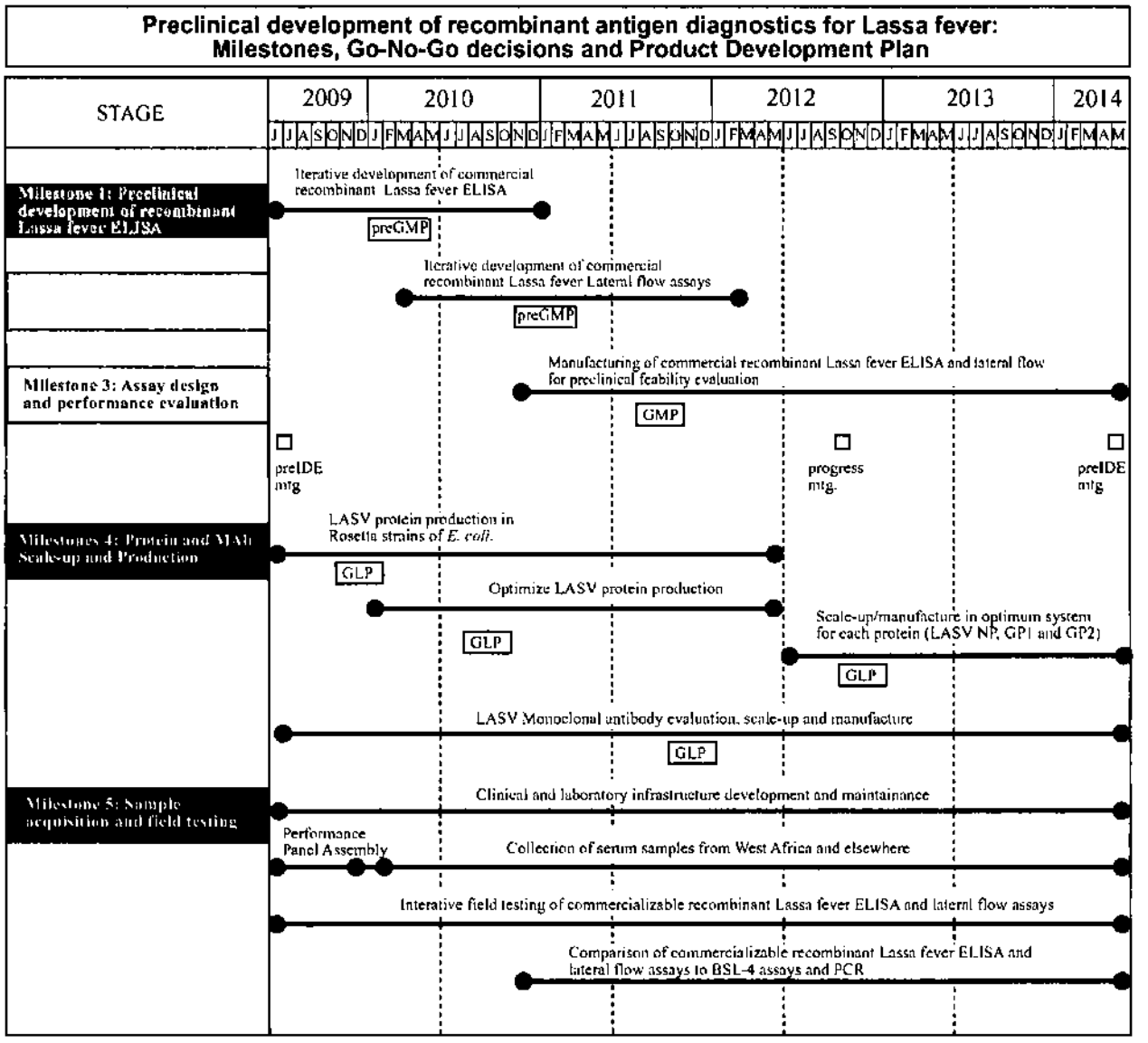

Fig. 10. Gantt Chart for preclinical development of commercial Lassa fever immunodiagnostics in a proposed 5 year project period. Milestones encompass one or more Go-No Go decisions.

specificity on the Performance Panel (Table 3), and we cannot increase the sensitivity/specificity, then we will consider that Milestone 2 has not been achieved. This assessment will be completed by the end of grant year 3. If a No Go decision is reached for Milestone 2B, then no additional funding for development of lateral flow assays will be accepted after year 3. Rather, we will renegotiate reduced levels of funding to pursue recombinant ELISA as the sole Lassa fever diagnostic platform.

Expected results, potential pitfalls and alternative approaches. We expect that the approaches taken will lead to Lassa fever recombinant lateral flow assays with sensitivities and specificities that are comparable to the BSL-4 and recombinant ELISA. Some modest reduction in these parameters as indicated will be considered acceptable. The ability for rapid diagnosis is particularly important in the case of Lassa fever given that the value of ribavirin treatment drops rapidly as the disease progresses. As is the case of the recombinant Lassa fever ELISA, lateral flow assays will be scalable, commercializable and not require BSL-4 facilities for production.

MILESTONE 3: Manufacturing of recombinant ELISA and lateral flow assays under GMP.
3A. Convert to manufacturing of recombinant ELISA and lateral flow assays GMP with QA)/QC to provide quantities of commercial grade diagnostic kits sufficient for preclinical evaluation of design control parameters to achieve benchmarks required for FDA approval.
Several design control parameters will be evaluated under Milestone 3:

- *Stability.* If positive samples remain positive and negative samples remain negative for 1 year from the date of manufacture, the kits will be considered to be stable and this will represent a Go decision for clinical valuation of the Lassa fever ELISA and lateral flow assays.
- *Temperature.* If similar assay results are produced over temperatures from 18 to 35°C, this will represent a Go decision for clinical valuation of the Lassa fever ELISA and lateral flow assays.
- *Precision.* If all precision measurements are ≤ 20%, this will represent a Go decision for clinical valuation of the Lassa fever ELISA and lateral flow assays.
- *Limit of Detection (LOD)/Limit of Quantitation (LOQ).* Go-No Go decision is not applicable.
- *Interference.* If none of the interfering substances cause greater than 20% difference from the results of samples without interferents in ELISA and if a false-positive or false-negative results are not obtained in the lateral flow format, this will represent a Go decision for clinical valuation of the Lassa fever ELISA and lateral flow assays.
- *Cross Reactivity.* If assay kits do not display cross reactivity with samples positive for other viral illnesses resulting in fever and other Lassa-like symptoms, such as malaria and typhoid, this will represent a Go decision for clinical valuation of the Lassa fever ELISA and lateral flow assays.
- *Linearity/Recovery.* If linear dilution range is obtained with patient samples from 1:10 to 1:2000, this will represent a Go decision for clinical valuation of the Lassa fever ELISA and lateral flow assays.
- *Sensitivity/Specificity.* Go-No Go decisions for this parameter are discussed in this section under Milestones 1, 2 and 5.

Expected results, potential pitfalls and alternative approaches. Failure in any parameter during the course of assay development will result in reformulation of the assays, including substitution of primary assay components (LASV proteins or mAbs), the addition of preservatives, stabilizers or other reformulations. If these assay adjustments are not successful for the ELISA, we will not accept further funding at that point in the project. If adjustments do not improve these parameters for the Lassa fever lateral flow assays, we will abandon this platform and focus on the recombinant ELISA, with renegotiation of the cooperative agreement to reflect the reduced scope of work. As discussed under the Product development plan, we also expect to have at lease two preIDE and one progress meeting or written exchanges with FDA personnel during the preclinical project period. Adverse guidance from FDA could result in No Go decisions during the project. If this occurs in any grant year, we will not accept further funding. We expect to be able to configure all the proposed Lassa fever ELISA and lateral flow assays within the targeted design control parameters. We do not anticipate that FDA guidance will result in any No Go decisions.

MILESTONE 4: Process development for production of recombinant LASV proteins and LASV - specific monoclonal antibodies.
4A. Optimize scale up/purification of recombinant LASV proteins, GP1, GP2, and NP.
A pertinent step in the development of commercial ELISA and lateral flow assays will be the production of commercially useful quantities of LASV NP, GP1 and GP2 and mAb to these proteins. This milestone is highly dependant on experimental results, and observations during the execution of this project will lead to alternate protein expression approaches. We will consider Milestone 4A to be achieved if we are successful in cloning and expressing LASV proteins with yield of 30 mg protein per liter of bacterial or mammalian cells (~3 fold current yields) for at least 2 of 3 of the recombinant LASV proteins. Yields of 20 mg protein per liter of bacterial or mammalian cells will be acceptable for 1 of 3 of the proteins. It may, for example, be difficult to express appropriately folded LASV GP2. We expect to make the determination of final manufacturing methods for each of the LASV recombinant proteins by the end of grant year 4. If these specified yields are not obtained by the end of year 4, we will not accept funding for year 5.

4B. Optimize scale up/purification of mAbs to recombinant LASV GP1, GP2, and NP.
Milestone 4B will be achieved if we are successful in developing sufficient number of scaled mAb producing cell lines (yields >15 mg/L) with a battery of different specificities for each of the LASV proteins to manufacture commercial Lassa fever ELISA and lateral flow assays. If adequate mAbs are not identified, or required cell lines prove resistant to scale-up, we will have reached a No-Go decision. We expect to be able to make this determination by the end of project year 4.

4C. Convert to manufacturing with QA/QC, to provide quantities of recombinant LASV proteins and mAbs sufficient for development and testing of commercial assays.
Preliminary data obtained to date with purified LASV proteins indicate that proposed diluents and formulation parameters will meet with success in the preparation of protein lots for the intended applications proposed herein. Reference standard and pre-clinical lots of recombinant protein that do not meet with acceptable criteria will not be used in studies and must either be reprocessed until specifications are met, or would result in the ultimate generation of a new protein lot from the cell culture or fermentation step onward.

Expected results, potential pitfalls and alternative approaches. These approaches will enable production of multi-milligram quantities of high quality LASV recombinant proteins and mAbs sufficient for development of prototype and commercial-grade arenavirus immunodiagnostics. We do not expect significant barriers to generating sufficient quantities of murine mAbs, which are not dependent on growth as ascites in mice for production.

MILESTONE 5: Field testing of commercial recombinant Lassa fever ELISA and lateral flow point-of-care diagnostics in West Africa.
5A. Define and collect positive and negative sera for assay validation from diverse regions across the Lassa fever endemic range of West Africa and elsewhere.
The collection of positive and negative sera positive and negative sera for assay validation from diverse regions across the Lassa fever endemic range of West Africa and elsewhere will proceed through out the project period. We will reach a No Go decision if target acquisition of samples fall below 50% of the targeted goal in any given grant year. If this occurs we will renegotiate a lower funding level dependent on NIAID objectives for this project.

Expected results, potential pitfalls and alternative approaches. We expect to meet target goals of sample acquisition, with the following caveats:

Skewed sampling toward severe cases. The samples to be tested in this study will be derived primarily from hospitalized patients. Although the sampling of a large number of patients over a broad expanse of West Africa should naturally result in representation of a wide spectrum of clinical disease, the sample is skewed toward disease syndromes severe enough for patients to seek medical care and, in most cases, be hospitalized. Mild and even asymptomatic Lassa fever have been described and may have a different antigen and antibody profile than that seen in symptomatic persons.

Logistical problems in maintaining the field work. Establishing and maintaining a high performance reliable laboratory in a developing country such as Sierra Leone and the other MRU countries is always a challenge. Maintenance of the cold chain where electrical supply is absent or inconsistence (and it is indeed inconsistent in Kenema) is especially problematic. The laboratory presently depends on and functions using town-supplied power, a series of generators, and solar-powered refrigerators and freezers. A battery bank and additional solar panels are scheduled for installation to further assure a stable supply of power. The team from Kenema, the other MRU-LFN country laboratories, and Tulane has over 10 years of experience working together to establish and maintain laboratories in West Africa, and is quite well-versed in confronting the challenges of this nature of field work.

Renewed civil instability in Sierra Leone or the other MRU-LFN countries. The MRU region has a history of political instability over the past two decades. Should civil unrest erupt, the ability to collect and test samples in the field could obviously be severely impaired. However, Sierra Leone, the country from where the most samples are likely to come, has become increasingly stable since the civil war in that country was declared over in 2002. Furthermore, the KGH Lassa ward remained functional even during the war and blood samples from suspected cases of Lassa fever were usually still taken. Thus, even in the scenario of significant civil unrest, clinical samples might still be available. Liberia is presently emerging from many years of civil war, but is also becoming increasingly stable with the aid of United Nations Peacekeeping Mission. Both Sierra Leone and Liberia undertook elections resulting in a peaceful transition of power in the last few years. While the region certainly has a long road to completely rebuild its society, infrastructure, and economy, there is no indication whatsoever of renewed violence. Considering that the acquisition of clinical samples is not completely tied to any one country, we are confident that the necessary clinical material can be obtained. In the event that laboratory testing is not possible in West Africa due to civil unrest or technical problems, the samples can, after inactivation, be sent for analysis at Tulane or USAMRIID.

5B. Test and compare the commercial Lassa fever recombinant ELISA and recombinant lateral flow point-of-care diagnostic assays with results from BSL-4 ELISA and PCR assays.
We expect that, the approaches taken will lead to Lassa fever ELISA assays and lateral flow assays with >85% sensitivity and >92% when tested on the clinical feasibility panel (Table 6). These values would equal that of current BSL-4 ELISA assays, with the added advantages that they are scalable, commercializable and do not require BSL-4 facilities for production. If these goals are meet we will reach a Go decision and proceed with further commercial development as discussed in the **Product Development Plan**.

Expected results, potential pitfalls and alternative approaches. It should be reiterated that an important goal of Milestone 5 will be to directly compare the newly derived commercializable assays with RT-PCR based assays. It is important to estimate the duration of a possible window phase early in infection, where RT-PCR is already positive but antigen assay may be still negative. Knowledge about this hypothetical window phase is relevant for interpretation of results obtained with the assays and management of patients. As discussed, we expected to obtain samples sequentially from patients at early stages in the disease, starting within a few days after development of other symptoms. This will allow us to precisely define the window phase, if it exists, comparing in this regard RT-PCR detection and the recombinant ELISA under development. It should be noted that PCR based assays were not as sensitive or specific as the Ag-capture assay (not visa versa) in the patient population tested in Preliminary Studies. Nevertheless, as part of our efforts we will attempt to improve detection of LASV by RT-PCR and will incorporate new primer sets of others to optimize paradigms for LASV detection in field-collected samples.

Patent status or other protection of project intellectual property.
A provisional patent application (Docket Number 12920.0018.PZUS00) was filed on April 10, 2007 with the following title and summary:
"SOLUBLE AND MEMBRANE-ANCHORED FORMS OF LASSA VIRUS SUBUNIT PROTEINS"
The present invention discloses compositions comprising soluble and membrane-anchored forms of Lassa virus (LASV) glycoprotein 1 (GP1), glycoprotein 2 (GP2), the glycoprotein precursor (GPC), and the nucleoprotein (NP). This invention also relates to diagnostic and preventative methods using the novel forms of the LASV subunit proteins. Preventative methods include preparation of vaccines, as well as factors (e.g. small molecules, peptides) that inhibit LASV infectivity. Further, the invention relates to diagnostic and therapeutic antibodies including neutralizing antibodies for the prevention and treatment of infection by LASV and other arenaviruses. The present invention also discloses and provides new tools and methods for the design, production, and use of soluble and membrane-anchored forms of LASV GP1, GP2, NP and GPC including expression in engineered bacterial- and mammalian-based systems.

Product Development Plan

Goals of the Project, including final products.
Our proposed project will accomplish preclinical development and validation of commercializable ELISA and lateral flow assays for Lassa fever. Six different assays will be developed and tested including:

- LASV antigen-capture ELISA
- LASV IgM-capture ELISA
- LASV IgG-capture ELISA
- LASV antigen-capture lateral flow assay
- LASV IgM-capture lateral flow assay
- LASV IgG-capture lateral flow assay

Following completing of the project, the ELISA and lateral flow assays for Lassa fever will then proceed to the next stages of product development for commercialization of the assays, including establishing the performance characteristics of the assay kits in clinical trials and PMA submissions.

The value of the product.
The assays will be used in conjunction for diagnosis of Lassa fever, with primary utility for bioterrorism scenarios. The assays will also have utility in clinical settings in West Africa where Lassa fever is endemic. LASV recombinant antigen diagnostics will provide significant value in two areas, biodefense and public health.

Development of a recombinant LASV diagnostics for biodefense will provide products that can:

- Diagnosis Lassa fever during deliberate relate of LASV, thus protecting civilian and military personnel, including first responders from secondary exposures.
- Diagnosis and distinguish attack with LASV from other potential bioterrrorism agents, including viruses causing other VHF.
- be stock-piled.
- be used to diagnosis accidental exposures to LASV in personnel working in BSL-4 laboratories.
- serve as a deterrent from using this easily acquired pathogen as a bioweapon.
- pilot diagnostic strategies, which may be useful against viruses causing other VHF that are potential bioweapons.

Development of recombinant LASV diagnostics for public health will provide a product that can:

- be used to diagnosis Lassa fever in persons living in endemic areas in West Africa after exposure to LASV carried by *Mastomys*.
- be used to protect persons traveling to endemic areas from exposure to LASV.
- be used to protect hospital personnel from exposure to LASV from infected patients.
- improve acceptance of health care leading to increases in hospital visits and decreasing fear of hospitals in LASV endemic areas.
- Be used in development of therapeutic, treatment and vaccines strategies against Lassa fever.
- Be used in studies of the natural history of Lassa fever.

Lay description of key technology objectives. The objective of this project is to create tests that can determine if a person has contracted Lassa fever, a serious, often fatal disease (hemorrhagic fever). Persons with Lassa fever may go into shock, develop failure of many organs in the body, and sometimes bleed from the lips, eyes, ears or other body openings. Lassa virus, which causes Lassa fever, is classified in the highest category of potential bioweapon threats, and is also of major public health importance in West Africa. Lassa virus is carried by a rodent ("rat"), which is abundant in West Africa and often spreads the virus to people. This virus would be very easy to obtain and disseminate, most likely by spraying it into the air where it would be inhaled. Some people exposed by breathing Lassa virus deliberately released into the air would be expected to develop Lassa fever. Because people in most developed countries rarely develop

hemorrhagic fevers such as Lassa, a cluster of cases would be a major public health incident. Our proposed project will further develop and validate two types of diagnostic assays for Lassa fever. The first type of assay, known as an ELISA (enzyme-linked immunoadsorbant assay), is a standard type of diagnostic assay. ELISA use relatively simple equipment found in every diagnostic laboratory in developed countries, as well as in most diagnostic laboratories in the developing world. ELISA are effective, rapid, highly sensitive and specific, not subject to false positive reactions due to laboratory contamination, easy to use, adaptable and cost-effective. A second type of assay we will develop to diagnosis Lassa fever is the lateral flow assay. This assay is similar to a home pregnancy test. Lateral flow assays are self-contained, and use no external equipment. Therefore, lateral flow assays can be used in emergency situations, such as after a bioterrorist attack with Lassa virus, or in resource-poor countries in west Africa where Lassa fever is prevalent. We will also develop safe and effective processes for producing the components of the diagnostic assays. The current diagnostic tests for Lassa fever use Lassa virus that must be grown in high containment ("space-suit") laboratories, and can only be produced in relatively small amounts in a highly labor-intensive effort. We will manufacture the Lassa proteins using bacterial, yeast or cultured animal cells in typical laboratories. The proteins are equivalent to the proteins found in Lassa virus, but are produced by DNA cloning. Because we will make the diagnostics with individual Lassa virus proteins that cannot be assembled into a live virus, the diagnostics will be safe to manufacture and use. The diagnostics for Lassa fever will be field-tested in a laboratory in Sierra Leone, the country that has the highest incidence of Lassa fever. The project will also perform many of the necessary steps that are required by the Food and Drug Administration (FDA) prior to marketing diagnostic tests, including providing data on assay reliability and stability.

Advantages compared to competing products, technologies, or services. Our prior experience with recombinant antigen diagnostic assays, our access to the BSL-4 facilities at USAMRIID, uniquely qualify our team to develop and validate recombinant antigen Lassa fever diagnostic assays. The LASV clinic where we work in Kenema, Sierra Leone, with its unique capabilities to diagnose LASV infection is the only such facility in the LASV endemic areas of West Africa, and indeed is the only such permanent facility dedicated to diagnosis and treatment a severe VHF. Thus, we are uniquely positioned to develop, validate and field test LASV in-vitro diagnostic (IVD) products. As discussed (Significance), currently available assays for Lassa fever use reagents that must be produced under BSL-4 conditions. Immunoassays remain the preferred method for diagnosis of infection with RNA viruses, such HIV and hepatitis C virus (HCV), rather than nucleic acid testing (NAT). In the case of HIV and HCV, NAT is either a clinical management tool or a confirmatory test for the diagnostic ELISA. Diversity amongst LASV isolates and the number of other arenaviruses that are potential bioterrorism agents suggests that development of useful NAT for arenaviruses will be technically challenging. It should be noted that in under-developed countries where arenaviruses are endemic and there are difficulties in maintaining a cold-chain ELISA may prove to be more robust than PCR (nucleic acids are of course less stabile than either viral antigens or virus-specific antibodies). Furthermore, PCR and other NAT methods require instrumentation, expertise and facilities generally not available in West Africa, which would effectively preclude clinical testing of standard NAT devices.

Competitive advantages of the proposed products versus BSL-4 based assays or NAT include:

- Increased safety profile—No live or attenuated virus.
- Scalable and consistent recombinant manufacturing process.
- Better long-term stability—longer shelf life.
- Point-of-care diagnosis (lateral flow assays)
- Effective
- Rapid
- specific, not subject to false positive reactions due to laboratory contamination
- easy to use
- adaptable
- cost-effective

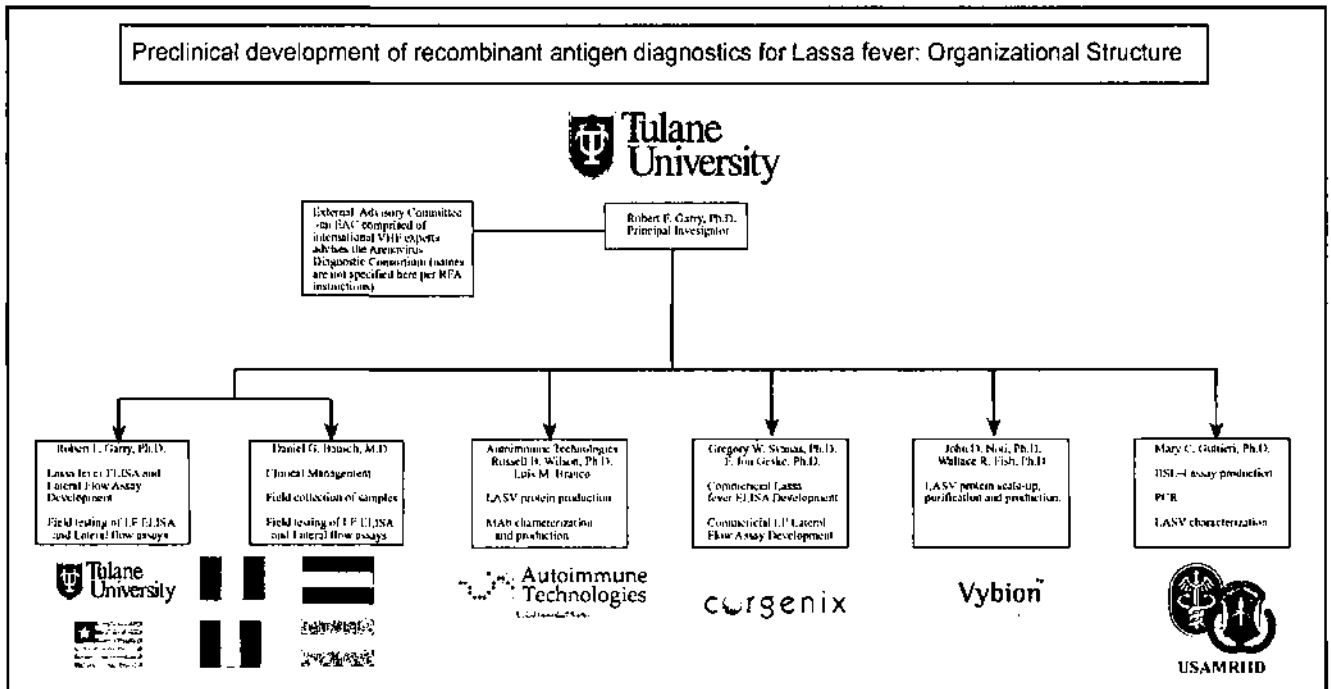

Fig. 11. Organizational Chart for preclinical development of commercial Lassa fever immunodiagnostics. Flags of Sierra Leone, Guinea, Liberia, Nigeria and Argentina represent countries from which serum samples will be obtained.

Critical path for further commercial development.

As defined by the FDA, the critical path for licensure of any medical device, including an *in vitro* diagnostic (IVD), can be broken down into five basic steps: 1) Basic Research; 2) Prototype Design or Discovery; 3) Preclinical Development; 4) Clinical Development; and 5) FDA Filing/Approval and Launch Preparation. Associated with the last four steps are three interdependent dimensions including Safety, Medical Utility, and Industrialization that incorporate step-specific tasks that must be completed for successful licensure. A further component of the critical path is appropriate and timely interactions with the FDA. In Milestones 1 through 5 of the proposed Experimental Plan, we have detailed the activities (Milestones) that encompass the step-specific tasks that are part of the critical path for FDA licensure of Lassa fever recombinant ELISA and lateral flow assays. The plan will bring Lassa fever recombinant Ag-, IgM and IgG-capture ELISA and lateral flow assays up to critical path step 4, Clinical Development. A graphical overview of the critical path up to FDA compliant clinical trials with associated timelines was provided in the prior section in Figure 10.

Precise role of each institution/company in the commercialization process and their interdependencies. The proposed project is intended to bring recombinant antigen IVD for Lassa fever through the preclinical evaluation phase. The Arenavirus Diagnostic Consortium is a uniquely experienced group of researchers from Tulane University, three private biotechnology companies and USAMRIID. There is excellent communication and cooperation amongst the investigators, who have meet 5 times as a group over the past three years in Frederick, MD, Denver, CO or New Orleans, LA for intensive planning/problem solving sessions. This planning will continue, with NIH Program staff input, under the proposed cooperative agreement.

The roles of each institution/company in the development and commercialization process for Lassa fever ELISA and lateral flow assays and their interdependencies are shown in diagrammatic form in Figure 11. To date the consortium has completed step 1 and 2 critical path activities for Lassa fever recombinant ELISA. Tulane University and USAMRIID scientists provided functional clones of the genes encoding LASV NP, GP1, GP2 and GPC to Luis Branco (then at BioFactura, now at Autoimmune). The LASV NP genes in bacteria and the glycoprotein were expressed in bacterial and/or mammalian cell lines and the purification parameters for the MBP fusion proteins were defined. The recombinant proteins were characterized by Mr. Branco in collaboration with Tulane and USAMRIID scientists (Drs. Joseph Fair and Mary Guttieri). The recombinant LASV NP, GP1, GP2 and GPC were used for production of screening ELISA for selection of murine hybridoma cell lines producing mAb to these LASV structural proteins. Using the recombinant proteins and murine mAb reagents, prototype Ag-capture, IgM- and IgG-capture ELISA have been developed and optimized by Corgenix, Autoimmune and Tulane scientists. ELISA-based prototype kits were manufactured to Autoimmune's specifications by Corgenix, the designated contract manufacturer. The research team at Tulane oversaw the acquisition of clinical samples and the field-testing of various prototype ELISA under development throughout the project period. Corgenix produced pilot lots of kits that were used for initial validation studies in Sierra Leone by Tulane scientists (Drs. Fair, Bausch and Garry).

The Consortium now proposes to complete step 3 of the critical path activities for Lassa fever recombinant ELISA. The Consortium also proposes to complete steps 1 to 3 of the critical path activities for Lassa fever lateral flow assays. After successful feasibility studies of both the ELISA and lateral flow assays in preclinical field studies, the Consortium will proceed with clinical testing and commercialization of the assays (step 4). Clinical trials will be conducted under Good Clinical Practices (GCPs) by an experienced partner or Contract Research Organization (CRO). PMA submissions and other activities will occur. The design and development, preclinical and clinical testing, manufacturing, labeling, distribution and promotion of commercial IVD products for use in human testing are subject to regulation by numerous governmental authorities, principally the United States Food and Drug Administration (FDA). In anticipation of eventual distribution of FDA-cleared Lassa diagnostics, assay development and final kit manufacturing at Corgenix will proceed under an FDA-specified design control system. Corgenix, who will manufacture the ELISA, has a quality system in place that exceeds the requirements of the FDA Quality System Regulation. In addition, the company holds EN/ISO 13485:2000 and ISO 13485:1996 (under CMDCAS) certification. Autoimmune Technologies, Corgenix, Vybion and their consultants are experienced in IVD, Medical Device and Pharmaceutical regulatory compliance and submissions, including pre-market approval (PMA) or 510(k) clearance submissions to the FDA. As product development efforts move to clinical safety trials, clinical manufacture of the diagnostics for human use must be performed under current Good Manufacturing Practices (cGMPs) described in Title 21 of the Code of Federal Regulations. cGMPs are mandated by the FDA to ensure that the products manufactured by the industries such as pharmaceutical, biotech and medical devices, meet specific requirements for identity, strength, quality, and purity.

Markets. We anticipate that there will be multiple commercial markets for Lassa fever diagnostic assays. The Lassa fever diagnostic assays will be principally useful for defense against bioterrorism scenarios in the United States and elsewhere. They would also be useful in clinical settings in West Africa and after adaptation for New World arenaviruses in South America. The advantages of ELISA-based diagnostics include their ease of standardization, widespread availability and applicability to the diagnosis of numerous other diseases. Lassa fever lateral flow assays will be valuable for rapid diagnosis during an incident of bioterrorism and could be used in technology poor regions such as West Africa. Potential sales of the products include US military and civilian agencies as well as international government agencies and public health institutions such as WHO. The US military acquires medical products to combat chemical and biological threats through the Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD). The US government has identified LASV as a biological agent that poses a high priority threat and the CDC have designated LASV a Category A biothreat. As the US agencies responsible for military and civilian biodefense acquire and adopt these Lassa fever diagnostic assays as a mainstay Lassa fever countermeasure, the equivalent agencies of allies around the world may also become customers. Recently,

NATO formed the multinational CBRN Defense Battalion to provide rapidly a credible Nuclear Biological Chemical (NBC) capability, primarily to deployed NATO joint forces and commands, in order that Alliance freedom of action is maintained in an NBC threat environment. This may be yet another customer of the Lassa fever diagnostic assays. Additionally, the diagnostic assays can also be sold to governments, hospitals, clinics and diagnostic laboratories in endemic regions such as West Africa. We anticipate that WHO, non-governmental organizations (NGO), and private foundations (for example the Gates Foundation) would provide supplemental markets for resource poor countries. We also anticipate that stockpiles of Lassa fever diagnostic assays for biodefense purposes could be transferred to endemic areas on a rotating basis, prior to shelf-life expiration.

Biosafety and Biocontainment

The Investigators will operate under all applicable Health and Human Services Department and Department of Agriculture regulations and Federal laws including the Patriot Act and the Tauzin Act. These requirements are designed to assure that potential bioterrorism agents and toxins are protected from loss and theft, and that personnel who work with them are trained, reliable and trustworthy.

Our biosecurity plan:

- Describes inventory control procedures, minimal education and experience criteria for those individuals with access to select agents or toxins, physical security, and cyber security;
- Contains provisions for routine cleaning, maintenance, and repairs; provisions for training personnel in safety and security procedures; provisions for securing the area;
- Describes procedures for loss or compromise of passwords, etc.;
- Contains procedures for reporting suspicious persons or activities, loss or theft of listed agents or toxins, release of listed agents or toxins, or alteration of inventory records;
- Contains provisions for the control of access to listed agents and toxins and safe operating procedures; and procedures for reporting and removing unauthorized persons;
- Contains provisions for ensuring that all individuals with access understand safety and security requirements and are trained and equipped to follow established safety and security procedures;
- Establishes procedures for reporting and removing unauthorized persons; and
- Establishes procedures for securing the area when approved individuals are not present.

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Protection of Human Subjects

Patients with febrile illnesses in West Africa

1. RISKS TO THE SUBJECTS

Specimens will be collected from patients suspected of having Lassa fever who present to one of the above-described facilities in West Africa. In the normal course of care of these patients blood will be drawn immediately upon clinical suspicion of an arenaviral hemorrhagic fever (which was most often at the time of admission) and then at various intervals depending on the clinical course. Once conventional ELISA testing for arenavirus infections is performed, the remaining serum will be available for testing with the ELISA in development. To guard patient confidentiality, diagnostic samples will be number-coded and all unique identifiers removed prior to the sample being made available to the research team for testing.

2. ADEQUACY OF PROTECTION AGAINST RISKS

Subjects are not directly recruited by Investigators in this study, but rather by collaborating investigators who follow approved consent procedures from their institution for drawing such blood samples. The study proposed here does not consist of a clinical trial and will not seek to obtain specimens that would not ordinarily be acquired through the clinically indicated management of the patient. Subjects will give consent that samples collected from them may be used for research. In no instance will subject identification be possible. There are no additional risks to subjects posed by the research proposed in this application. There is complete protection of the confidentially of subjects as no identifying information is associated with the blood samples.

3. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

There will be no direct benefits to subjects of this research. However, to insure optimal management of the patient and the safety of the medical staff, with the consent of the hospital administration, patient isolation procedures and facilities will be reviewed for each center and revised or up-graded as necessary. Training sessions in infection control will be conducted and an adequate supply of personal protective equipment and other necessary supplies for the optimal care of patients with Lassa fever, such as syringes and intravenous fluids, will be supplied to each hospital. With the consent of the laboratory directors, the laboratory infrastructure and operating procedures will be reviewed and revised or up-graded as necessary. Training sessions for the laboratory technicians and personal protective equipment will be supplied to each laboratory.

4. IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

The potential use of LASV as a biological weapons directed against civilian or military targets necessitates development of effective diagnostics. The Lassa fever diagnostic immunoassays would be used to determine the attack agent following a deliberate release, and allow the virus used to be distinguished from other hemorrhagic fever viruses, such as dengue virus or Ebola virus, that may have similar case presentations. Development of rapid immunodiagnostic assays will also improve treatment of Lassa fever, facilitate studies to understand their prevalence and natural history, and ultimately lead to vaccines for preventing these a major cause of morbidity and mortality. We will also gain important knowledge regarding the utility of development and prototype assays to diagnose VHF.

Group II: Healthy blood donors

1. RISKS TO THE SUBJECTS

We will obtain blood from healthy donors to validate the immunoassays under development. Other than the pain of a venipuncture there is minimal risk in giving blood. There is a risk that confidentially could be compromised.

2. ADEQUACY OF PROTECTION AGAINST RISK

We will obtain IRB approval to obtain this blood and use an institutional approved written consent procedure. To guard patient confidentiality, diagnostic samples will be number-coded and all unique identifiers removed prior to the sample being made available to the research team for testing.

3. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

We pay blood donors as an inducement to participate. There are no benefits to donors other than payment as an inducement. Identities of donors are confidential except to the extent that our financial services department write out checks to each blood donor.

4. IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

We will gain important knowledge regarding the utility of development and prototype assays to diagnose LASV infection.

Inclusion of women and minorities

The gender of the patients is not selected and no particular minority group is targeted or excluded. Blood donors are not selected on the basis of sex or ethnic background. Minorities in the study population will be recruited proportionally to their prevalence in the United States population. Prisoners or other institutionalized individuals are not used for this study. Women of childbearing potential will not be excluded.

Targeted/Planned Enrollment Table

Study Title: Preclinical development of recombinant antigen diagnostics for Lassa fever

Total Planned Enrollment: 6220

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	75	75	150
Not Hispanic or Latino	3035	3035	6070
Ethnic Category: Total of All Subjects *	3110	3110	6220
Racial Categories			
American Indian/Alaska Native	2	2	4
Asian	2	2	4
Native Hawaiian or Other Pacific Islander	1	1	2
Black or African American	2605	2605	5210
White	500	500	1000
Racial Categories: Total of All Subjects *	3110	3110	6220

* The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: Total of All Subjects."

Inclusion of children
Children will be included in the analysis of patients suspected of having Lassa. Children (i.e., individuals under the age of 21) are not included as healthy blood donors as their inclusion would not meaningfully contribute to the study.

Animal Use
Animals were used under IACUC approval in preliminary studies to generate mAbs and polyclonal sera against recombinant LASV proteins. We believe that the 130 MAbs producing cell lines and goat sera raised to date are sufficient to proceed successfully with commercial Lassa fever immunoassay development. Therefore, no animal studies are proposed in the current application. Should our experimental results indicate that additional mAbs or polyclonal sera are necessary (for example: to detect divergent LASV isolates), we will obtain IACUC approval to produce these reagents. No animal studies will be performed without full IACUC review.

Select Agent Research
Because of Biosecurity issues associated with LASV culture, all experiments involving replication-competent arenaviruses will be performed in the secure BSL-4 laboratories at USAMRIID. Patient specimens will not leave the BSL-4 laboratories in the field sites in Africa or South America unless they have been autoclaved, subjected to sterilizing doses of γ irradiation, exposed to chemical agents to extract viral nucleic acids and destroy viral infectivity, or packed and shipped directly to USAMRIID in secure containers by approved couriers. All work with replication-competent LASV or other arenaviruses will be performed at a containment level of BSL-4 by personnel certified for work at BSL-4. The BSL-4 suites of USAMRIID are located at Fort Detrick, and are secured by United States military personnel. All established protocols for handling virus, animals, and "sharps" at BSL-4 will be followed. The infectious agent employed in this protocol will be LASV, strain Josiah, registration #643 for BSL-4 Clinical Laboratory, #8007 in AA-4, and #291 in AA-5. Any sera samples, or reagents produced during the course of this study that may contain live virus will be irradiated (if required) and thoroughly safety-tested before removal from BSL-4 containment. Persons with access to biological select agents used in this protocol are listed on the Centers for Disease Control and Prevention Form 4B of Alan Schmaljohn, Connie Schmaljohn, and Cindy Rossi.

Consortium/Contractual Arrangements
A CRDA will be signed between Tulane University and USAMRIID regarding the current project.

Letters of Support
Letters of Support follow from:

- Col. George W. Korch, Jr., Commanding Officer USAMRIID
- Humarr Khan, MD. Chief of the Lassa Ward and Augustine Goba, Director Lassa Laboratory, Kenema Government Hospital, Kenema, Sierra Leone
- Mamadou Coulibaly, Director, Tulane-International Center for Research on Tropical Infections (Tulane-CIRIT), N'Zérékoré, Guinea
- Delia Enria, MD, Director, Instituto National de Enfermedades Virales, Pergamino, Argentina
- Jerry Dubai, Laboratory Director, Central Public Health Laboratory Service at JFK Hospital, Ministry of Health, Monrovia, Liberia
- George O. Akpede, FWACP, Irrua Specialist Teaching Hospital, Nigeria
- Professor Robert Swanepoel, Special Pathogens Unit, National Institute for Communicable Diseases, Sandringham, South Africa
- Clarence J. Peters, MD, University of Texas Medical Branch at Galveston, Texas.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES
1425 PORTER STREET
FORT DETRICK, MARYLAND 21702-5011

REPLY TO
ATTENTION OF:

June 12, 2008

Business Plans and Programs Office

Robert F. Garry, Ph.D.
Assistant Dean, Graduate Program in Biomedical Sciences
Tulane University
1430 Tulane Avenue, SL-38
New Orleans, Louisiana 70112-2699

Dear Dr. Garry:

The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) encourages its scientists to collaborate with investigators from other institutions on National Institutes of Health (NIH) and National Institute of Allergy and Infectious Diseases (NIAID) funded grants and contracts. Such collaborations allow USAMRIID to leverage technology and resources from other organizations in order to achieve its mission. Tulane University (Tulane) is planning to submit by June 19, 2008 a grant application titled: Pre-clinical Development of Recombinant Antigen Lassa Fever Diagnostics, UO1 RFA-AI-08-001. This grant will be in collaboration with one of our lead scientists, Dr. Mary C. Guttieri. There are no actual or potential conflicts of interest for this proposed effort and Dr. Guttieri's supervisor approves of this partnership.

USAMRIID will 1) generate Biosafety Level 4 reagents; 2) assist with assay development; 3) conduct comparative investigations between traditional and newly-engineered assays; and 4) assist with implementation/testing of assays at the Lassa Fever Laboratory in Kenema, Sierra Leone.

Since USAMRIID does not have the administrative resources to manage these NIH-funded contracts, we will utilize the T.R.U.E. Research Foundation (T.R.U.E.) for the administration of these contracts. T.R.U.E. is a not-for-profit corporation established specifically to administer funds received from external sources for use by the military medical community.

Once the grant is funded, we would like to propose that a three-way Cooperative Research and Development Agreement (CRADA) be negotiated between USAMRIID, T.R.U.E. and Tulane for execution of the work to be conducted at USAMRIID. T.R.U.E.'s role will strictly be administrative in nature and their participation will have no impact on the fulfillment of the Statement of Work. We believe that this is the best

mechanism for ensuring accountability required by both NIH/NIAID and Tulane for management of these contracts.

In the event of a national defense priority and subject to availability of resources, and negotiated cost sharing with Tulane, USAMRIID reserves the right to delay, modify, or withdraw from this agreement. If the proposal is resubmitted, a new letter of intent will be required.

My point of contact to this initiative is Mr. Dan Coffman, Business Plans and Programs Office, at 301-619-6886 or email at james.coffman@amedd.army.mil.

Sincerely,

for George W. Korch, Jr.
Colonel, U.S. Army
Commanding

LASSA FEVER LABORATORY
KENEMA GOVERNMENT HOSPITAL
KENEMA, SIERRA LEONE
Tel: +232.076.757.859

Robert F. Garry, PhD
Professor, Department of Microbiology and Immunology
Tulane Health Sciences Center
1430 Tulane Ave., SL-38
New Orleans, Louisiana 70112-2699
Email: rgarry@tulane.edu
Fax: (504) 988-1998

May 29, 2008

Dear Dr. Garry:

We are writing to express our enthusiastic support for your application for NIH RFA-AI-08-003, *Cooperative Research Partnerships for Biodefense and Emerging Infectious Diseases (U01)*. As you know, the problem of Lassa fever is of much more than academic interest in Sierra Leone, where the disease is a real possibility in virtually every febrile patient. Diagnosing and distinguishing Lassa fever from the many other causes of febrile syndromes is thus a major concern. Your proposed project entitled *Preclinical development of recombinant antigens for the diagnosis of Lassa fever* unites a uniquely experienced group of researchers from academia, industry and the United States military with the goal of further developing and validating ELISA-based assays for Lassa fever. We were partners in the initial stages of this work, which have been very promising, and are more than happy to again collaborate with you to see this diagnostic format through to commercial availability. The plan to develop a lateral flow rapid test format is of particular interest and would be of great use here in Sierra Leone.

The Lassa Laboratory in Kenema (established in 2004 with the support of Tulane and the World Health Organization through the Mano River Union Lassa Fever Network), the long-standing presence of a ward uniquely dedicated to the care of patients with Lassa fever (where up to 300 cases may be seen yearly, from whom samples will be available), and the cadre of experienced researchers represented in your research proposal offer an unparalleled combination of resources to mount a project such as this. Indeed, it is hard to conceive of this project being possible anywhere else in the world. Furthermore, the continued development of research infrastructure and partnerships in Sierra Leone will form an excellent basis for future projects and scientific advances in Lassa fever, with the potential for studies on prevention, immune response to infection, and therapeutic and vaccine candidates.

Testing and transfer of all samples for this and any future projects will, of course, comply with all applicable laws and regulations on the Select Agents. We at the Lassa Laboratory and Lassa Ward at Kenema Government Hospital look forward to another productive collaboration with you and your team.

Very sincerely,

LASSA FEVER LABORATORY
KENEMA GOVERNMENT HOSPITAL
KENEMA, SIERRA LEONE

Augustine [illegible]
Director
Lassa Laboratory
Kenema Government Hospital
Kenema, Sierra Leone
Phone: 232 76 757 859
Email: [illegible]

Sheik Humarr Khan
Chief Physician
Lassa Ward
Kenema Government Hospital
Kenema, Sierra Leone
Phone: 232 33 630 524
Email: [illegible]

Jun 09 / 06 / 2008

Robert F. Garry, PhD
Professor, Department of Microbiology and Immunology
Tulane Health Sciences Center
1430 Tulane Ave., SL-38
New Orleans, Louisiana 70112-2699
Fax: (504) 988-1994

Dear Dr. Garry,

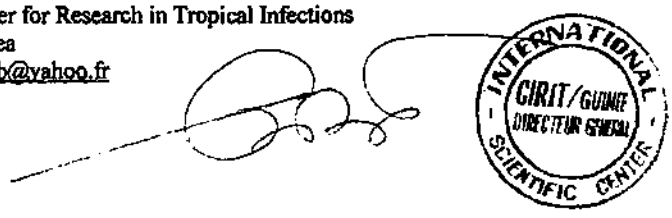
I am writing to express my support for your NIH application "Preclinical development of recombinant antigens for the diagnosis of Lassa fever." Lassa fever is endemic in Guinea. In addition, the potential use of arenaviruses as biological weapons directed against civilian or military targets necessitates development of effective diagnostics. Development of rapid immunodiagnostic assays will also improve treatment of arenaviral diseases, facilitate studies to understand their prevalence and natural history, and ultimately lead to vaccines to prevent these major causes of morbidity and mortality.

The International Center for Research in Tropical Infections (French acronym: CIRIT) is located on the grounds of N'Zérékoré Regional Hospital, a major reference hospital in the heart of the Lassa-endemic area of Guinea. CIRIT has had a productive collaboration with Tulane University since 1996, most of it focused on Lassa fever. We will again be happy to collaborate with you, including, among other activities, making clinical samples from patients with Lassa fever available for testing. Testing and transfer of all samples will, of course, comply with all applicable laws and regulations of both Guinea and the United States.

I look forward to an exciting collaboration and productive work with you on this timely and innovative research project.

Very sincerely,

Mamadou Coulibaly, PhD
Director
International Center for Research in Tropical Infections
N'Zérékoré, Guinea
Email: coulinterlab@yahoo.fr



Ministerio de Salud
Secretaría de Políticas, Regulación e Institutos
ADMINISTRACION NACIONAL
DE LABORATORIOS E INSTITUTOS DE SALUD
"DR. CARLOS G. MALBRAN"
Instituto Nacional de Enfermedades Virales Humanas
"Dr. Julio I. Maiztegui"

Pergamino, June 17, 2008.

Robert F. Garry, PhD
Professor
Department of Microbiology and Immunology
Tulane Health Sciences Center
1430 Tulane Ave., SL-38
New Orleans, Louisiana 70112-2699
Fax: (504) 988-1994

Dear Dr. Garry,

I am writing to express my support for your NIH grant entitled *Preclinical development of recombinant antigens for the diagnosis of Lassa fever*. The further development and validation of the recombinant antigen-based assays that you and your team have developed are important steps toward having a commercially available assay—a critical need for both biodefense and public health. In the development of these assays, it will be important to assess the degree of cross reactivity against other arenaviruses. Toward that end, I would like to offer support by making available serum samples from my institution from persons infected with the arenavirus Junin virus in Argentina. I look forward to working with you on this important research project.

Sincerely,

Delia A. Enria, MD, MPH
Director
Instituto Nacional de Enfermedades
Virales Humanas "Dr. J.I. Maiztegui"
Monteagudo 2510 (2700) Pergamino
Argentina
Tel: 54-2477-433044 int 200
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E- mail: direccioninevh@speedy.com.ar

REPUBLIC OF LIBERIA
MINISTRY OF HEALTH & SOCIAL WELFARE
P O. BOX 10 - 9009
1000 MONROVIA 10, LIBERIA
WEST AFRICA

Ref No.

June 6, 2008

Robert F. Garry, PhD
Professor, Department of Microbiology and Immunology
Tulane Health Sciences Center
1430 Tulane Ave., SL-38
New Orleans, Louisiana 70112-2699
Email: rgarry@tulane.edu
Fax: (504) 988-1994

Dear Dr. Garry:

Thank you for the invitation to participate in your NIH grant entitled *Development of recombinant antigens for the diagnosis of Lassa fever* in response to NIH RFA-AI-08-001, *Collaborative Research Partnerships for Biodefense and Emerging Infectious Diseases* (U01). Lassa fever is major public health problem in Liberia, with frequent suspected or confirmed cases (total more than 80 in 2007), even with inadequate surveillance. Even unconfirmed cases result in significant expenditure of time, money and effort, to remove these valuable resources away from the many other health needs of the Liberian population. Therefore, we look very favorably on any project designed to enhance diagnostic capability for Lassa fever. In the past year, Liberia has been profiting from its membership in the Mano River Union Lassa Fever Network, which assists countries in our region to build capacity for containing Lassa fever and other highly communicable diseases. The project, which is led by the World Health Organization and implemented primarily by Tulane University, New Orleans, LA, has re-established ELISA-based diagnostics for Lassa fever in neighboring Sierra Leone. We frequently send samples from suspected cases in Liberia to this laboratory and are very satisfied with the turn-around and quality of the diagnosis rendered. However, we must note that Lassa fever probably frequently occurs in remote rural regions of Liberia, and the cases that we are able to suspect, obtain serum from, and send to Sierra Leone for diagnosis probably represent a small fraction of the total. Clearly, diagnostic testing for Lassa fever needs to be more readily available in Liberia. The development of a bed-side rapid test format, as called for in your grant, would be particularly useful, and indeed would drastically change the "landscape" of our knowledge of Lassa fever in Liberia. We are happy to participate in the project and look forward to working with you.

Sincerely,

Jerry Dahn
Laboratory Director
Central Public Health Laboratory Service at JFK Hospital
Ministry of Health
Government of Liberia
Monrovia, Liberia
Phone: +231 5623268
Email: jgdahn@yahoo.com

MOH
LABORATORY

IRRUA SPECIALIST TEACHING HOSPITAL

P.M.B. 6, IRRUA,
EDO STATE, NIGERIA.

Chief Medical Director
George O. Akpede,

UP

17th June, 2008

Robert F. Garry, PhD
Professor, Dept. of Microbiology & Immunology
Tulane Health Services Centre
1430 Tulane Ave SL-38
New Orleans Louisiana 70112-2699
E-mail rfgarry@tulane.edu
Fax: (504)988-1994

Dear Prof. Garry,

RE: EXPRESSION OF INTEREST IN YOUR RESEARCH PROJECT

I write to express support and interest in your NIH U01 Cooperative Research Grant proposal entitled *Preclinical development of recombinant antigens for the diagnosis of Lassa fever.* This project got to our knowledge through Dr. Daniel Bausch of Tulane School of Public Health and Tropical Medicine.

2. Whereas Lassa fever remains a significant public health problem in Nigeria, the dearth of laboratory diagnostics poses a major challenge to case recognition and treatment as well as the collection of reliable surveillance data. It is also an impediment to the establishment of effective control measures.

3. Our institution is in the epicenter of the epidemic in Nigeria. Partly for this reason, and partly by virtue of our membership of the Lassa Fever Stakeholders' Forum of Nigeria, we have been very interested in furthering efforts in Lassa fever research and control activities.

4. We were therefore quite pleased to know about your project and shall be willing to assist in the research, including making aliquots of samples from suspected cases of Lassa fever available to your research team. This is because we believe that the project has tremendous potential to advance the efforts in prevention and control of Lassa fever.

5. We urge you to kindly accept our warm greetings.

George O. Akpede, FWACP
Professor of Paediatrics and
Chief Medical Director

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

NICD

Practice No. 5200768

Phone: 27 11 386 - 6335
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Internet : http//www.nicd.ac.za

(Switchboard: 27 11 386-6000)

Private Bag X4
Sandringham 2131
South Africa

18 June 2008

Dr RF Garry
Department of Tropical Medicine
Tulane School of Public Health and Tropical Medicine
New Orleans LA 70112
USA

Dr Garry
Project to validate and standardize recombinant Lassa fever diagnostic antigen

This letter is to confirm our willingness to participate in your project to validate and standardize recombinant Lassa fever antigen by facilitating the obtaining of access to 2,500 control sera from healthy South African persons.

Regards

Professor Robert Swanepoel
Special Pathogens Unit, NICD

UTMB
The University of Texas Medical Branch

C.J. Peters, M.D.
John Sealy Distinguished University Chair in Tropical & Emerging Virology

June 14, 2007

Robert F. Garry, PhD
Professor
Department of Microbiology and Immunology
Tulane Health Sciences Center
1430 Tulane Ave., SL-38
New Orleans, Louisiana 70112-2699
Fax: (504) 988-1994

Dear Dr. Garry,

I am writing to express my enthusiastic support for your NIH U01 Cooperative Research Grant proposal entitled *Preclinical development of recombinant antigens for the diagnosis of Lassa fever.* The potential use of arenaviruses as biological weapons directed against civilian or military targets necessitates development of effective diagnostics. The proposed project unites a uniquely experienced group of researchers from academia, industry and the United States military, building upon their previous successful work in developing recombinant-based antigens. The project ultimately has the potential not only to result in a commercially available product, but also a rapid test format. Such products would be invaluable in detecting, controlling, and preventing the transmission of Lassa and other arenaviruses, be it through intentional release as a bioweapon, exposure to the natural rodent reservoirs, or secondary person-to-person transmission. The diagnostic assay to be developed will allow infection with Lassa virus to be detected and distinguished from other viral hemorrhagic fevers, allow for more rapid treatment, and facilitate studies to understand the prevalence and natural history of transmission. Diagnostics assays are also essential in developing vaccines to prevent these major causes of morbidity and mortality.

Although the target disease for the assays is Lassa fever, it will also be important to validate them for the South American arenaviruses (Junin, Machupo, Guanarito, or Sabiá viruses). In order to achieve this, I will be happy to make laboratory generated sera and clinical samples from patients infected with South American arenaviruses that might be available through my laboratory available to you. Testing and transfer of all samples will, of course, comply with all applicable laws and regulations on the select agents. I look forward to an exciting collaboration and productive work with you on this timely and innovative research project.

Wishing you the best of luck in your endeavors,

Sincerely,

C. J. Peters, M.D.

CJP:ljv

G.170 Keiller Building, 301 University Boulevard, Galveston, TX 77555-0609
(409)772-0090 tel., (409)747-0762 fax. cjpeters@utmb.edu

Obtaining and Disseminating Biomedical Research Resources

Tulane University recognizes the necessity to balance the protection of intellectual property rights with the need to broadly disseminate new discoveries. Autoimmune will follow the Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources (64 FR 72090) for: 1. Minimizing administrative impediments to the exchange of biomedical research tools; 2. Ensuring academic freedom and timely disclosure of research findings; 3. Ensuring appropriate implementation of the Bayh-Dole Act; and 4. Ensuring dissemination of research resources developed with NIH funds. Tulane University pledges to coordinate its efforts with those of the other subcontractors and collaborators under such a contract to: i) protect intellectual property arising in the performance of the contract; ii) facilitate the development for commercialization of the resulting therapeutic product; and iii) resolve disputes among the collaborating parties should such disputes arise in the performance of the contract. Specifically in these regards, Autoimmune will ensure that intellectual property resulting from this program is protected and developed appropriately in full compliance with the NIH guidelines and principles. Tulane University will ensure that research materials developed during the work activities of this program will be shared by all parties involved on terms essentially identical to those preferred by the NIH. Autoimmune will work to further develop and commercialize resulting products. Tulane University will serve as the primary mediator among collaborating parties and will provide, to the best of its abilities, a neutral forum for resolution of any disputes.

Sharing Research Data (Plan)

Data sharing will be accomplished through 1) Publishing - We will publish articles in scientific publications fully describing our results, and 2) Researchers' Efforts-we will respond directly to data requests by mailing a CD-ROM containing data or by posting data on our Web site (All the Virology on the World Wide Web; www.virology.net). Data will be compiled and posted in Excel spreadsheets, which are accessible to all researchers. Data sharing will be initiated in a timely manner, no later than the acceptance for publication of the main findings from the final dataset. We will not place limits on questions or methods, nor require co-authorship as a condition for receiving data. We will also provide proper documentation to ensure that others can use the dataset and to prevent misuse, misinterpretation, or confusion. We are also aware that the Office of Management and Budget (OMB) Circular A-110 has been revised to provide public access to research data through the Freedom of Information Act (FOIA) that are supported in whole or in part with Federal funds. Upon approval of clinical trial protocols by the NIAID, the will be submitted to ClinicalTrials.gov using the Protocols Registration System as mandated by HR3580, Food and Drug Administration Amendments Act of 2007)

Sharing of Model Organisms for Biomedical Research (Plan)

Bacterial and mammalian cell lines producing recombinant LASV proteins derived in the course of this work will be made accessible to the general scientific community. Bacterial and mammalian cells expressing LASV proteins will be deposited with the American Type Culture Collection and will be provided to verifiably legitimate users for non-commercial uses without restriction.

Grant Number: 5U01AI082119-05 REVISED

Principal Investigator(s):
Robert F Garry, PHD

Project Title: Preclinical development of recombinant antigen diagnostics for Lassa fever

DIRECTOR, RESEARCH ADMIN
TULANE UNIV HLTH SCIENCES CTR
1430 TULANE AVENUE
NEW ORLEANS, LA 70112

Award e-mailed to: elecnotf@tulane.edu

Budget Period: 04/01/2013 – 03/31/2014
Project Period: 04/22/2009 – 03/31/2014

Dear Business Official:

The National Institutes of Health hereby revises this award to reflect an increase in the amount of $52,641 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to TULANE UNIVERSITY OF LOUISIANA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 31 USC 6305 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number U01AI082119. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F. Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation; until then, Awardees must comply with the 1995 regulation. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Laura C. Eisenman
Grants Management Officer

Additional information follows

SECTION I – AWARD DATA – 5U01AI082119-05 REVISED

Award Calculation (U.S. Dollars)

Salaries and Wages	$173,322
Fringe Benefits	$35,044
Supplies	$56,400
Travel Costs	$23,500
Patient Care (Inpatient)	$47,000
Other Costs	$48,733
Consortium/Contractual Cost	$688,023
Federal Direct Costs	$1,072,022
Federal F&A Costs	$165,130
Approved Budget	$1,237,152
Federal Share	$1,237,152
TOTAL FEDERAL AWARD AMOUNT	$1,237,152
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$52,641

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
5	$1,237,152	$1,237,152

Fiscal Information:

CFDA Number:	93.855
EIN:	1720423889A1
Document Number:	UAI082119A
Fiscal Year:	2013

IC	*CAN*	*2013*
AI	8472315	$1,237,152

NIH Administrative Data:
PCC: M32B B / **OC**: 414P / **Released**: LEISENMAN 06/12/2013
Award Processed: 06/13/2013 12:10:55 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5U01AI082119-05 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5U01AI082119-05 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

Carry over of an unobligated balance into the next budget period requires Grants Management Officer prior approval.

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/#gps .

A final Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 90 days of the expiration date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) cash transaction data.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 90 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm.

Unless an application for competitive renewal is submitted, a final progress report must also be submitted within 90 days of the expiration date. Instructions for preparing a Final Progress Report are at: http://grants.nih.gov/grants/funding/finalprogressreport.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the final progress report. Institute/Centers may accept the progress report contained in competitive renewal (type 2) in lieu of a separate final progress report. Contact the awarding IC for IC-specific policy regarding acceptance of a progress report contained in a competitive renewal application in lieu of a separate final progress report.

NIH **strongly encourages** electronic submission of the final progress report and the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final progress report and final invention statement may be e-mailed as PDF attachments to the NIH Central Closeout Center at: DeasCentralized@od.nih.gov.

Hard copy: Paper submissions of the final progress report and the final invention statement may be faxed to the NIH Division of Central Grants Processing at 301-480-2304, or mailed to:

NIH Division of Central Grants Processing, OER
6705 Rockledge Drive
Suite 5016, Room 5109
MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express mail delivery only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final Progress Report is not required. However, a final FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5U01AI082119-05 REVISED

REVISED AWARD: This award was revised to restore funding near or at NIAID Funding Plan Levels, and consistent with NIH FY2013 Fiscal Policy per NOT-OD-13-064, http://grants.nih.gov/grants/guide/notice-files/NOT-OD-13-064.html.

Supersedes Notice of Award issued 3/26/2013.
****

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.
****

Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at http://www.selectagents.gov/Regulations.html) must complete registration with CDC (or APHIS,

depending on the agent) before using NIH funds. No funds can be used for research involving Select Agents if the final registration certificate is denied.

Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS.
****

This award is subject to the Terms and Conditions of Award as set forth in the SPECIAL REQUIREMENTS section of RFA-AI-08-001, NIH Guide to Grants and Contracts, 02/21/2008. These special terms and conditions are incorporated in this award by reference.

Copies of the RFA may be accessed at the following Internet address:
http://www.nih.gov/grants/guide/index.html

As mandated in this RFA, the principal investigator should submit a performance plan to the NIAID program official that details specific milestones and timelines for achieving each milestone. Milestones should be linked to the annual funding cycle and submission of the annual progress report. The plan should include the specific criteria to be used in evaluating the degree of progress made in achieving each milestone.

The timelines and milestones must be approved by the Program Official within 30 days from the issue date of award.

Such timelines and milestones shall be agreed upon by the principal investigator and the NIAID program official before funds may be released.

To receive consideration for funding of each successive year, the annual progress report and an Updated Product Development Plan must be received two months prior to the end of the current funding period, demonstrating that the milestones defined for that funding year have been met.

The award may be adjusted in time or funding, as necessary, if the grantee fails to meet the agreed upon milestones.
****

This award includes funds awarded for consortium activity with Autoimmune Technologies, LLC, Corgenix Medical Corporation and Vybion. Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at
http://grants.nih.gov/grants/policy/nihgps_2010/nihgps_ch15.htm#_Toc271265266, pages IIB-232-236.
****

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Tina M. Carlisle
Email: carlislt@niaid.nih.gov **Phone**: 301-402-6579 **Fax**: 301-493-0597

Program Official: Patricia M. Repik
Email: prepik@niaid.nih.gov **Phone**: 301-451-3504 **Fax**: 301-480-1594

SPREADSHEET SUMMARY
GRANT NUMBER: 5U01AI082119-05 REVISED

INSTITUTION: TULANE UNIVERSITY OF LOUISIANA

Budget	***Year 5***

Salaries and Wages	$173,322
Fringe Benefits	$35,044
Supplies	$56,400
Travel Costs	$23,500
Patient Care (Inpatient)	$47,000
Other Costs	$48,733
Consortium/Contractual Cost	$688,023
TOTAL FEDERAL DC	$1,072,022
TOTAL FEDERAL F&A	$165,130
TOTAL COST	$1,237,152

Facilities and Administrative Costs	***Year 5***
F&A Cost Rate 1	49%
F&A Cost Base 1	$336,999
F&A Costs 1	$165,130

Progress Report Scanning Cover Sheet

5U01AI082119-05

PI Name:	**GARRY, ROBERT**
Org:	**TULANE UNIVERSITY OF LOUISIANA**
Start Date:	**04/01/2013**
Snap:	**N/A** (NEEDS TO BE BOOKMARKED)
Appl ID:	**8458569**
Rec'd Date:	**02/21/2013**

Form Approved Through 06/30/2012 OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Progress Report

Review Group	Type	Activity	Grant Number
ZAI1MMTMJ1	5	U01	5U01 AI082119-~~04~~ 05

Total Project Period
From: 04/22/2009 Through: 03/31/2014

Requested Budget Period
From: 04/01/2013 Through: 03/31/2014

1. TITLE OF PROJECT
Preclinical development of recombinant antigen diagnostics for Lassa fever

2a. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR
(Name and address, street, city, state, zip code)
Garry, Robert F.
Tulane University
Department of Microbiology and Immunology
1430 Tulane Avenue

2b. E-MAIL ADDRESS
rfgarry@tulane.edu

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Microbiology and Immunology

2d. MAJOR SUBDIVISION
School of Medicine

2e. Tel: (504) 988-2027 Fax: (504) 988-1994

3a. APPLICANT ORGANIZATION
(Name and address, street, city, state, zip code)
Tulane University
6823 St. Charles Avenue
New Orleans, LA 70118

3b. Tel: Fax:

3c. DUNS: 053785812

4. ENTITY IDENTIFICATION NUMBER
1720423889A1

5. NAME, TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL
Kathleen M. Kozar
Director, Sponsored Projects Administration
Tulane University HSC; New Orleans LA 70112

Tel: (504) 988-5613 Fax: (504) 988-1748

E-MAIL: elecnotf@tulane.edu

6. HUMAN SUBJECTS ☐ No ☒ Yes

6a. Research Exempt	If Exempt ("Yes" in 6a): Exemption No.	If Not Exempt ("No" in 6a): IRB approval date
☒ No ☐ Yes		Jan 19, 2012

6b. Federal Wide Assurance No. FWA00002055

6c. NIH-Defined Phase III Clinical Trial ☒ No ☐ Yes

7. VERTEBRATE ANIMALS ☒ No ☐ Yes

7a. If "Yes," IACUC approval Date

7b. Animal Welfare Assurance No.

8. COSTS REQUESTED FOR NEXT BUDGET PERIOD

8a. DIRECT $ 1,140,450 8b. TOTAL $ $1,316,120

9. INVENTIONS AND PATENTS ☒ No ☐ Yes

If "Yes, ☐ Previously Reported
☐ Not Previously Reported

10. PROJECT/PERFORMANCE SITE(S)

Organizational Name: Tulane University of Louisiana

DUNS: 053785812

Street 1: Tulane University

Street 2: 6823 St. Charles Avenue

City: New Orleans	County: Orleans
State: Louisiana	Province:
Country: United States	Zip/Postal Code: 70118

Congressional Districts: 2

11. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION *(Item 13)*
Kathleen M. Kozar Director, Sponsored Projects Administration

TEL: (504) 988-5613 FAX: (504) 988-1748 E-MAIL: elecnotf@tulane.edu

12. Corrections to Page 1 Face Page

13. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 11. *(In ink)* [signature]

DATE 2-19-13

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert F.

DETAILED BUDGET FOR NEXT BUDGET PERIOD – DIRECT COSTS ONLY	FROM 4-1-13	THROUGH 3-31-14	GRANT NUMBER 1U01AI082119-03

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Robert Garry, PhD	PI	EFFORT			30,117	5,512	35,629
John Schieffelin, MD	Investigator				13,575	2,430	16,005
Allyson Haislip	Lab Manager				30,103	8,880	38,983
Lina Moses	Program Coordinator				30,283	8,934	39,217
Chris Bishop	Program Manager				20,375	6,011	26,386
Personal Info	Graduate Student				33,682	3,099	36,781
	Graduate Student				26,250	2,415	28,665
SUBTOTALS →					184,385	37,281	221,666

CONSULTANT COSTS	0
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)*	60,000
TRAVEL	25,000
INPATIENT CARE COSTS	50,000
OUTPATIENT CARE COSTS	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	0
OTHER EXPENSES *(Itemize by category)*	51,844
SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD	**$ 408,510**
CONSORTIUM/CONTRACTUAL COSTS – DIRECT COSTS	614,853
CONSORTIUM/CONTRACTUAL COSTS – FACILITIES AND ADMINISTRATIVE COSTS	117,087
TOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD *(Item 8a, Face Page)*	**$ 1,140,450**

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert F.

DETAILED BUDGET FOR NEXT BUDGET PERIOD – DIRECT COSTS ONLY	FROM 4-1-13	THROUGH 3-31-14	GRANT NUMBER 1U01AI082119-05

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Russell B. Wilson	Director, Subcontract	EFFORT			15,451	3,863	19,314
Peter C. Kulakosky	Investigator				18,540	4,635	23,175
Luis M. Branco	Investigator				53,304	13,326	66,630
Stephanie McCormick	Lab Supervisor				25,000	6,250	31,250
Stephanie Gross	Laboratory Technician II				11,847	2,962	14,809
Milele Ramsay	Laboratory Technician II				36,057	9,014	45,071
SUBTOTALS					160,199	40,050	200,249

CONSULTANT COSTS	0
EQUIPMENT *(Itemize)*	0
SUPPLIES *(Itemize by category)* Bacterial fermentation and mammalian cell culture supplies. Appropriate resins, dedicated columns, and accessories for protein purification	38,577
TRAVEL Funds are requested to support travel of Dr. Wilson and Mr. Blanco to the facilities of Vybion or Corgenix for review and coordination of efforts.	4,000
INPATIENT CARE COSTS	0
OUTPATIENT CARE COSTS	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	0
OTHER EXPENSES *(Itemize by category)*	0
SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD	**$ 242,826**
CONSORTIUM/CONTRACTUAL COSTS – DIRECT COSTS	0
CONSORTIUM/CONTRACTUAL COSTS – FACILITIES AND ADMINISTRATIVE COSTS	0
TOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD *(Item 8a, Face Page)*	**$ 242,826**

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert F.

DETAILED BUDGET FOR NEXT BUDGET PERIOD – DIRECT COSTS ONLY	FROM 4-1-13	THROUGH 3-31-14	GRANT NUMBER 1U01AI082119-05

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Kelly R Pitts, PhD	Director, Subcontract	EFFORT			40,559	10,140	50,699
Matt Boisen	R&D Staff				32,909	8,227	41,136
Darin Oottamasathien	R&D Staff				33,743	8,436	42,179
Abby Jones	R&D Staff				34,414	8,604	43,018
Dan Simpson	Regulatory Affairs				6,089	1,522	7,611
Bethany Belote	Quality Assurance				10,730	2,683	13,413
SUBTOTALS →					158,444	39,612	198,056

CONSULTANT COSTS	0
EQUIPMENT *(Itemize)*	0
SUPPLIES *(Itemize by category)* Supplies both for ELISA microplate development and lateral flow (rapid) product development.	52,884
TRAVEL Bi-annual meetings with Tulane and other development partners to discuss progress and future	4,000
INPATIENT CARE COSTS	0
OUTPATIENT CARE COSTS	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	0
OTHER EXPENSES *(Itemize by category)*	0
SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD	$ 254,940
CONSORTIUM/CONTRACTUAL COSTS – DIRECT COSTS	0
CONSORTIUM/CONTRACTUAL COSTS – FACILITIES AND ADMINISTRATIVE COSTS	0
TOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD *(Item 8a, Face Page)*	$ 254,940

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert F.

DETAILED BUDGET FOR NEXT PERIOD – DIRECT COSTS ONLY - VYBION	FROM 4-1-13	THROUGH 3-31-14	GRANT NUMBER 1U01AI082119-05

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Lee Henderson, PhD	Director, Subcontract	EFFORT			26,250	7,613	33,863
Hui Zhu, MS	Scientist				38,522	11,171	49,693
SUBTOTALS					64,772	18,784	83,556

CONSULTANT COSTS	0
EQUIPMENT *(Itemize)*	0
SUPPLIES *(Itemize by category)* Bacterial fermentation and mammalian cell culture supplies. Appropriate resins, dedicated columns, and accessories for protein purification	30,000
TRAVEL Funds are requested to support travel of Dr. Henderson to the facilities of AIT or Corgenix for review and coordination of efforts.	3,531
INPATIENT CARE COSTS	0
OUTPATIENT CARE COSTS	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	0
OTHER EXPENSES *(Itemize by category)*	0
SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD	**$** 117,087
CONSORTIUM/CONTRACTUAL COSTS – DIRECT COSTS	0
CONSORTIUM/CONTRACTUAL COSTS – FACILITIES AND ADMINISTRATIVE COSTS	0
TOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD *(Item 8a, Face Page)*	**$** 117,087

BUDGET JUSTIFICATION	GRANT NUMBER 1U01AI082119-04

Provide a detailed budget justification for those line items and amounts that represent a significant change from that previously recommended. Use continuation pages if necessary.

Tulane University

Personal Info graduate student EFFORT months) replaces Personal Info graduate student EFFORT

Autoimmune Technologies

Stefanie McCormick effort reduced from EFFORT calender months

Corgenix

Dan Simpson effort reduced from EFFORT calendar months

Vybion

Brian Corbin no longer with project

Lee Henderson effort increased from EFFORT calendar months

Hui Zhu effort increased from EFFORT calendar months

CURRENT BUDGET PERIOD	FROM	THROUGH

Explain any estimated unobligated balance (including prior year carryover) that is greater than 25% of the current year's total budget.

not applicable

PHS 398/2590 OTHER SUPPORT

GARRY, ROBERT F.

ACTIVE

1U01AI082119 Garry (PI) 06/01/09 - 05/31/14 EFFORT
NIAID $7,073,538
Preclinical development of recombinant antigen diagnostics for Lassa fever
The goal is to complete the preclinical development phase for recombinant IgM-, IgG- and antigen-capture assays and point-of-care lateral flow assays for diagnosis of infection by Lassa virus. Role: PI
Role: PI

HHSN272200900049C (PI-Robinson) 09/30/09 – 09/29/14 EFFORT
NIAID-DAIT $7, ,280,388
Roles of protective or pathogenic B cell epitopes in human Lassa fever
The goals are to discover novel B cell epitopes of Lassa virus protein antigens and to elucidate mechanisms of antibody-mediated protection or pathogenesis in a well-characterized cohort of persons exposed to diverse strains of LASV at different stages and with different severities of Lassa fever.
Role: Program Manager responsible for overall scientific and logistical management of the project.

HHSN272201000022C (Co-PIs: Sabeti and Garry) 10/01/10 – 09/30/15 EFFORT
NIAID $7,, 88,85,
Host Genetic Factors in Resistance to Lassa Hemorrhagic Fever
The goals of this Project are to replicate strong signals of natural selection found in Yorubans at genes critical for infection with Lassa virus (LASV) in four additional West African populations and to localize and characterize the key functional mutations.
Role: Director of subcontract.

PENDING

Pending Support

OVERLAP

Dr. Garry will not accept total funding over 11.4 calendar months.

BRANCO, LUIS M.

ACTIVE

1U01AI082119 Garry (PI) 06/01/09 - 05/31/14 EFFORT
NIAID $7,073,538
Preclinical development of recombinant antigen diagnostics for Lassa fever
The goal is to complete the preclinical development phase for recombinant IgM-, IgG- and antigen-capture assays and point-of-care lateral flow assays for diagnosis of infection by Lassa virus. Role: PI
Role: Invesigator

HHSN272200900049C (PI-Robinson) 09/30/09 – 09/29/14 EFFORT
NIAID-DAIT $7, ,280,388
Roles of protective or pathogenic B cell epitopes in human Lassa fever
The goals are to discover novel B cell epitopes of Lassa virus protein antigens and to elucidate mechanisms of antibody-mediated protection or pathogenesis in a well-characterized cohort of persons exposed to diverse strains of LASV at different stages and with different severities of Lassa fever.
Role: Invesigator

PENDING

Pending Support

OVERLAP

Dr. Branco will not accept total funding over 12.0 calendar months. He will reduce effort on HHSN272200900049C, if 1R01AI104621 is funded.

HENDERSON, LEE A.

ACTIVE

1U01AI082119 Garry (PI) 06/01/09 - 05/31/14 EFFORT
NIAID $7,073,538
Preclinical development of recombinant antigen diagnostics for Lassa fever
The goal is to complete the preclinical development phase for recombinant IgM-, IgG- and antigen-capture assays and point-of-care lateral flow assays for diagnosis of infection by Lassa virus. Role: PI
Role: Director of Vybion subcontract

PENDING

None

OVERLAP

None

KULAKOSKY, PETER C

ACTIVE

1U01AI082119 Garry (PI) 06/01/09 - 05/31/14 EFFORT
NIAID $7,073,538
Preclinical development of recombinant antigen diagnostics for Lassa fever
The goal is to complete the preclinical development phase for recombinant IgM-, IgG- and antigen-capture assays and point-of-care lateral flow assays for diagnosis of infection by Lassa virus. Role: PI
Role: Investgator

PENDING
None

OVERLAP
None

PITTS, KELLY R.

ACTIVE

1U01AI082119 Garry (PI) 06/01/09 - 05/31/14 EFFORT
NIAID $7,073,538
Preclinical development of recombinant antigen diagnostics for Lassa fever
The goal is to complete the preclinical development phase for recombinant IgM-, IgG- and antigen-capture assays and point-of-care lateral flow assays for diagnosis of infection by Lassa virus. Role: PI
Role: Investgator

HHSN272200900049C (PI-Robinson) 09/30/09 – 09/29/14 EFFORT
NIAID-DAIT $7, ,280,388
Roles of protective or pathogenic B cell epitopes in human Lassa fever
The goals are to discover novel B cell epitopes of Lassa virus protein antigens and to elucidate mechanisms of antibody-mediated protection or pathogenesis in a well-characterized cohort of persons exposed to diverse strains of LASV at different stages and with different severities of Lassa fever.
Role: Investigator

PENDING
None

OVERLAP
None

SCHEIFFELIN, JOHN C.

ACTIVE

1U01AI082119 Garry (PI) 06/01/09 - 05/31/14 EFFORT
NIAID $7,073,538
Preclinical development of recombinant antigen diagnostics for Lassa fever
The goal is to complete the preclinical development phase for recombinant IgM-, IgG- and antigen-capture assays and point-of-care lateral flow assays for diagnosis of infection by Lassa virus. Role: PI
Role: Investigator

HHSN272200900049C (PI-Robinson) 09/30/09 – 09/29/14 EFFORT
NIAID-DAIT $7, ,280,388
Roles of protective or pathogenic B cell epitopes in human Lassa fever
The goals are to discover novel B cell epitopes of Lassa virus protein antigens and to elucidate mechanisms of antibody-mediated protection or pathogenesis in a well-characterized cohort of persons exposed to diverse strains of LASV at different stages and with different severities of Lassa fever.
Role: Investigator

HHSN272201000022C (Co-PIs: Sabeti and Garry) 10/01/10 – 09/30/15 EFFORT
NIAID $7,, 88,85,
Host Genetic Factors in Resistance to Lassa Hemorrhagic Fever
The goals of this Project are to replicate strong signals of natural selection found in Yorubans at genes critical for infection with Lassa virus (LASV) in four additional West African populations and to localize and characterize the key functional mutations.
Role: Investigator

PENDING
None

OVERLAP
None

WILSON, RUSSELL B.

ACTIVE
1U01AI082119 Garry (PI) 06/01/09 - 05/31/14 EFFORT
NIAID $7,073,538
Preclinical development of *recombinant antigen diagnostics for Lassa fever*
The goal is to complete the preclinical development phase for recombinant IgM-, IgG- and antigen-capture assays and point-of-care lateral flow assays for diagnosis of infection by Lassa virus. Role: PI
Role: Investigator

HHSN272200900049C (PI-Robinson) 09/30/09 – 09/29/14 EFFORT
NIAID-DAIT $7, ,280,388
Roles of protective or pathogenic B cell epitopes in human Lassa fever
The goals are to discover novel B cell epitopes of Lassa virus protein antigens and to elucidate mechanisms of antibody-mediated protection or pathogenesis in a well-characterized cohort of persons exposed to diverse strains of LASV at different stages and with different severities of Lassa fever.
Role: Investigator

PENDING
None

OVERLAP
None

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert F.

PROGRESS REPORT SUMMARY	GRANT NUMBER 1U01AI082119-04	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Robert F. Garry	FROM 04-01-12	THROUGH 03-31-13

APPLICANT ORGANIZATION
Tulane University

TITLE OF PROJECT (*Repeat title shown in Item 1 on first page*)
Preclinical development of recombinant antigen diagnostics for Lassa fever

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	☒ No Change Since Previous Submission	☐ Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	☒ No Change Since Previous Submission	☐ Change
C. Select Agent Research	☒ No Change Since Previous Submission	☐ Change
D. Multiple PD/PI Leadership Plan	☒ No Change Since Previous Submission	☐ Change
E. Human Embryonic Stem Cell Line(s) Used	☒ No Change Since Previous Submission	☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

A. SPECIFIC AIMS (MILESTONES)

MILESTONE 1: Development of commercial Lassa fever ELISA.
1A. Optimize commercial grade Lassa virus (LASV) antigen-capture and immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody-capture enzyme-linked immunosorbant assays (ELISA) using a Performance Panel of well-characterized sera.
1B. Convert to manufacturing of ELISA under Good Manufacturing Practices (GMP) with Quality Assurance (QA)/Quality Control (QC) to provide quantities of commercial grade diagnostic kits sufficient for preclinical evaluation of design control parameters.

MILESTONE 2: Development of Lassa fever lateral flow point-of-care diagnostics.
2A. Develop LASV antigen-, IgM- and IgG-capture lateral flow assays.
2B. Validate the lateral flow assays as point-of-care diagnostics using confirmed Lassa fever clinical samples and control samples field-collected from Guinea, Sierra Leone, Liberia and Nigeria, and compare lateral flow assay sensitivity and specificity to ELISA.

MILESTONE 3: Process development for production of recombinant Lassa virus proteins and Lassa virus -specific monoclonal antibodies.
3A. Optimize scale up/purification of recombinant LASV proteins, GP1, GP2, and NP.
3B. Optimize scale up/purification of monoclonal antibodies to recombinant LASV GP1, GP2, and NP.
3C. Convert to manufacturing with QA/QC, to provide quantities of recombinant proteins and monoclonal antibodies sufficient for development and testing of commercial assays.

MILESTONE 4: Field testing of commercial Lassa fever ELISA and lateral flow point-of-care diagnostics in West Africa.
4A. Determine preclinical feasibility of the commercial grade antigen-capture and antibody-capture LASV ELISA using confirmed Lassa fever clinical samples and control samples to achieve benchmarks required for FDA approval.
4B. Evaluate and compare LASV ELISA, lateral flow point-of-care diagnostics and polymerase chain reaction assay performance using clinical isolates from diverse regions in the Lassa fever endemic range and areas of West Africa that have high and low prevalences of Lassa.
4C. Develop and perform preclinical testing of algorithms for optimal diagnosis of Lassa fever in bioterrorism and public health scenarios.

B. STUDIES AND RESULTS

MILESTONE 1: Development of commercial Lassa fever ELISA.
1A. Optimize commercial grade LASV antigen-capture and immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody-capture enzyme-linked immunosorbant assays (ELISA) using a Performance Panel of well-characterized sera.

We have developed commercial Lassa fever (LF) antigen-, IgM- and IgG-capture assays.

1B. Convert to manufacturing of ELISA under Good Manufacturing Practices (GMP) with Quality Assurance (QA)/Quality Control (QC) to provide quantities of commercial grade diagnostic kits sufficient for preclinical evaluation of design control parameters.

Design control of the IgM, IgG and Ag-capture ELISA is nearly complete. GMP Lots of ELISA plates and LFIs ar being prepared on a routine basis.

MILESTONE 2: Development of Lassa fever lateral flow point-of-care diagnostics.
2A. Develop LASV antigen-, IgM- and IgG-capture lateral flow assays.

A POC lateral flow immunoassays (LFI) for LF has been developed which has a high sensitivity and specificity in Sierra Leonean isolates.

2B. Validate the lateral flow assays as point-of-care diagnostics using confirmed Lassa fever clinical samples and control samples field-collected from Guinea, Sierra Leone, Liberia and Nigeria, and compare lateral flow assay sensitivity and specificity to ELISA.

Rapid test validation has been performed on the LF LFI. Key points are summarized in Figures 1-7.

- Rapid test range is 0.0025 to 10µg/ml of LASB NP (Fig. 1).
- Linear range of LFI as 0.005 to 0.5 µg/ml of LASV NP (Fig. 2)
- Rapid Test Cut-off is [0.005µg/mL] which is equivalent to [150pg/test] Precision at cut-off: ratio 0.06±0.017 =m CV=28% (Fig. 3)
- Analytical precision of the rapid tests is within design goals (CV<20%) (Fig. 4)
- Precision of clinical samples within range of assay varies between 10 – 35% CV
- Precision of clinical samples is within design goals of between 10-35% CV (Fig. 5).
- US Normals give extremely low background on LFI and no false positives in over 200 samples tested (Fig. 6).

The Lassa fever LFI is performing at levels that meet or exceed comparable commercial diagnostics.

MILESTONE 3: Process development for production of recombinant Lassa virus proteins and Lassa virus -specific monoclonal antibodies.

3A. Optimize scale up/purification of recombinant LASV proteins, GP1, GP2, and NP. *Bacterial LASV NP, GP1 and GP2 scale up/purification.*

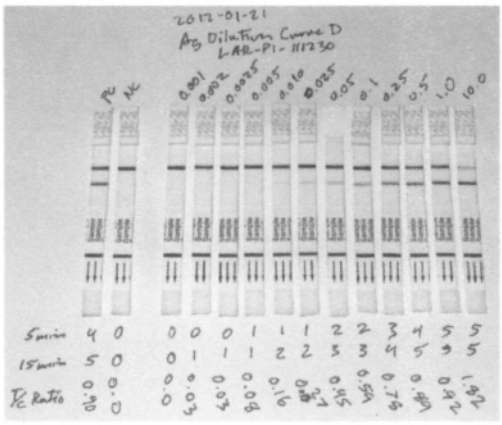
As described in previous updates, the production and purification of LASV LNP/GP1/GP2 from *E.coli* was successfully transferred to Vybion.

3B. Optimize scale up/purification of monoclonal antibodies to recombinant LASV GP1, GP2, and NP.

We have developed a novel technology for generating stable cell lines for production of our MAbs.

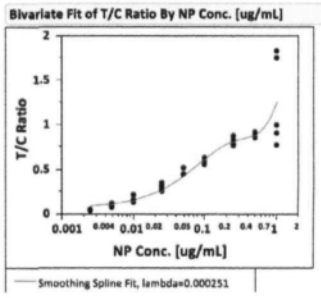
Expression Vectors: Light and Heavy chain antibody genes from MAbs 19.7E and 10.4B were re-engineered with optimal Kozak sequences and deconvolved 5' UTRs, and cloned in dual mammalian expression vectors, in tandem and in opposing orientations (see Fig. 7). In transiently transfected HEK-293T/17 cells opposing orientation gene constructs resulted in higher secreted antibody levels than from tandem counterparts, for both 19.7E and 10.4B. NS0

Rapid Test Range: 0.0025 to 10ug/mL NP in Hu Sera



Performance Testing in LFL, Kenama, SL

Small Prozone Effect on Control Line seen above [1µg/mL] does not affect Test Line signal development.



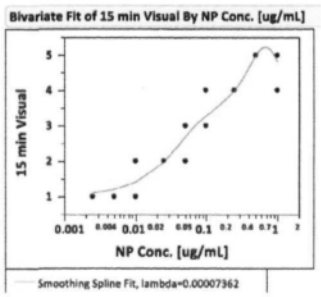
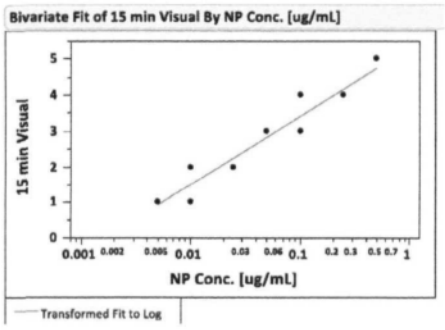
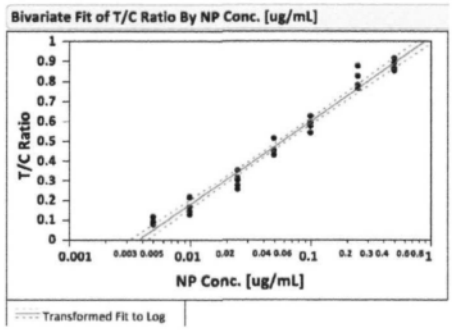


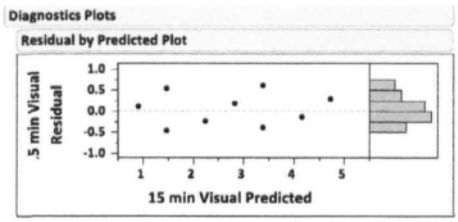
Figure 1. Detection range of Lassa fever Lateral flow immunoassay.

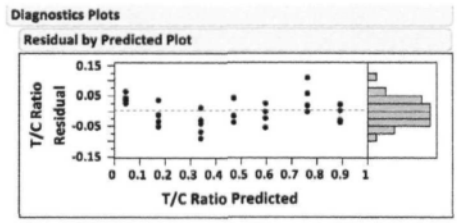
Linear Range: 0.005 to 0.5ug/mL NP in Hu Sera

Same range for Visual Read and Reflectance Ratio (n=5 at each level)







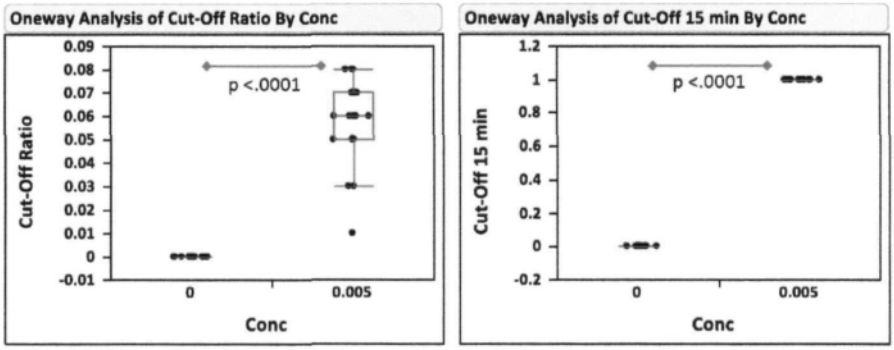


*same data set for both analysis

Figure 2. Linear range of Lassa fever Lateral flow immunoassay.

Rapid Test Cut-off Determination

LASV NP diluted in Hu Serum Control vs Hu Serum Control.
(30µL/dipstick, Control N=20, Cut-off sample N=27)

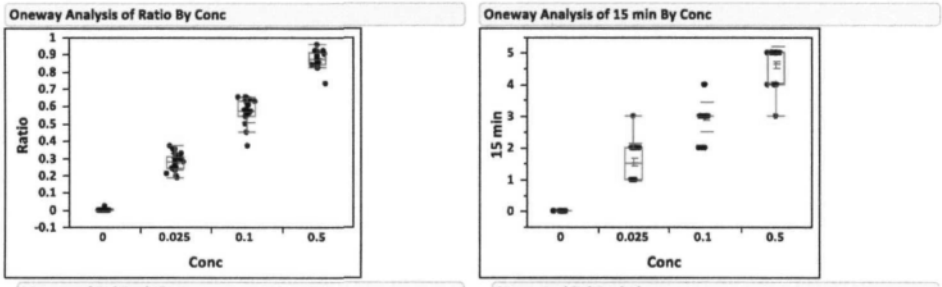


Rapid Test Cut-off is [0.005µg/mL] which is equivalent to [150pg/test].

Precision at Cut-off: Ratio (mV) = 0.060±0.017 (SD, N=27), CV = 28%

Figure 3. Lassa fever Lateral flow immunoassay cut-off.

Precision within Linear Range



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0	24	0.000833	0.004082	0.00083	-0.0009	0.00256
0.025	24	0.277500	0.049366	0.01008	0.2567	0.29835
0.1	24	0.569583	0.065374	0.01334	0.5420	0.59719
0.5	24	0.869583	0.046670	0.00953	0.8499	0.88929

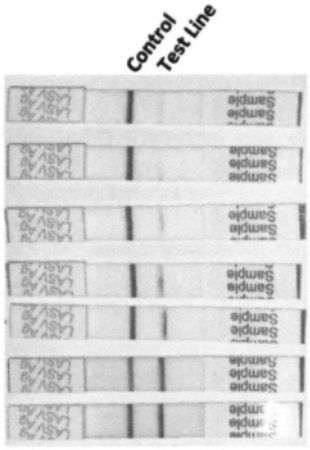
Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0	24	0.00000	0.000000	0.00000	0.0000	0.0000
0.025	24	1.54167	0.588230	0.12007	1.2933	1.7901
0.1	24	2.95833	0.464306	0.09478	2.7623	3.1544
0.5	24	4.62500	0.575779	0.11753	4.3819	4.8681

Analytical Precision of Rapid Test is with in Design Goals (CV <20%) however, the more relevant precision characterization is with clinical samples.

Figure 4. Precision within linear range of Lassa fever Lateral flow immunoassay.

Precision of Clinical Samples



	Ratio Precision	Visual Precision	
G-1905	0.11 ± 0.04	1.0 ± 0.5	(SD, N=8)
G-1794	0.19 ± 0.07	1.3 ± 0.4	(SD, N=16)
G-2273	0.33 ± 0.11	2.0 ± 0.3	(SD, N=8)
G-1762	0.42 ± 0.10	2.6 ± 0.5	(SD, N=16)
G-2222	0.56 ± 0.13	2.8 ± 0.7	(SD, N=8)
G-2190	0.97 ± 0.11	4.8 ± 0.5	(SD, N=8)
G-2263	0.93 ± 0.03	5.0 ± 0.3	(SD, N=16)
	0.00 – 1.00 mV	0.0 – 5.0 Score	(Ranges)

Precision of clinical samples within range of assay varies between 10 – 35% CV

Figure 5. Precision of Lassa fever Lateral flow immunoassay on clinical samples.

US Normals Specificity Panel

(Off the Clot Serums from Corgenix QC Specificity Panel)

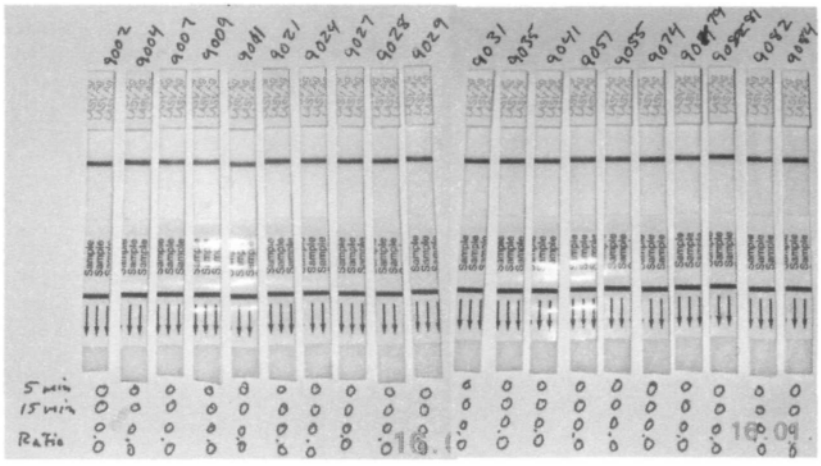


Figure 6. Performance of range of Lassa fever Lateral flow immunoassay on US Normals.

cell lines expressing 19.7E and 10.4B were generated by transfection with opposing antibody gene constructs.

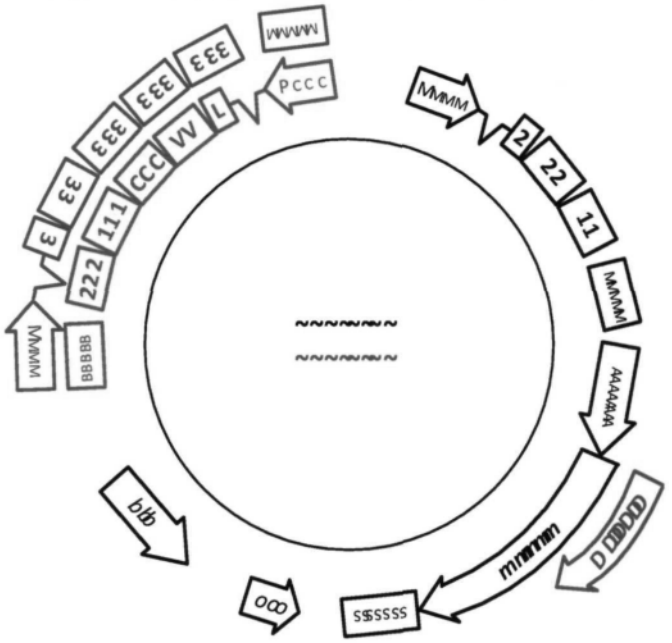


Fig. 7. Diagram of light and heavy chain antibody constructs in mammalian expression vectors containing a murine dhfr (ms dhfr) or hydroxysteroid dehydrogenase (msHSD) selectable markers

Stable NS0 cell lines: CholCelect, a novel mammalian expression system (U.S. Patent 8,076,102) designed for the generation of stable NS0 cell lines, that employs a cholesterol pathway rescue metabolic selection marker (NS0 cells are cholesterol auxotrophs), resulted in the generation of suspension and serum-free medium adapted clonal lines in approximately 2 months. Stable NS0 lines with specific productivity rates (SPR) ≥ 30 pg/cell/day, with volumetric productivity of greater than 110 mg/L were obtained with this system (Table 1 and 2). These volumetric yields were obtained in static T-flask cultures, without optimization. Such high levels of basal antibody expression commonly accommodate volumetric yields greater than 1g/L upon basic design-of-experiment (DOE) medium optimization experiments in shaker flasks. Currently, formulation optimization experiments are underway to permit scale-up and purification of multi-gram levels of each antibody from spinner flasks.

Stable CHO cell lines: Tandem and opposing orientation 19.7E constructs expressing the dihydrofolate reductase (dhfr) gene marker were transfected into CHO DG44 cells (dhfr-/-), and selected with different levels of methotrexate (MTX). Screening and characterization of cell lines will commence shortly. Initial ELISA screening of supernatants from emerging CHO DG44 lines transfected with a dual 19.7E huMAb construct identified a substantial number of high expressing isolates. These cell lines will be expanded and subjected to amplification with the selective dhfr inhibitor MTX to augment copy number and expression levels of 19.7E MAb. Generation of CHO DG44: 10.4B cell lines will follow.

3C. Convert to manufacturing with QA/QC, to provide quantities of recombinant proteins and monoclonal antibodies sufficient for development and testing of commercial assays.

All component of the assays are now being manufactured to industry standards of QC/QA which we believe will meet benchmarks required for FDA approval.

Table 1. Characterization of stable NS0:10.4B cell lines

Cell Line	Seed Density	Final Density	Final Viability	10.4B (µg/ml)	Doubling Time (hours)	Productivity (pg/cell/day)	
NS0_C.60	N.0_C06	N.N_C06	NN0	NN0	N0._	N6	N80_C.
NS0__.0N	N.0_C06	0.N_C06	NN0	N6	NN.0	6	
NS0__.N6	N.0_C06	0.0_C06	NN0	NN	N0.6	N	
NS0__.N6	N.0_C06	N.N_C06	NN0		NN.N	NN	N80_C.
NS0_C.66	N.0_C06	0.N_C06	NN0		NN.0	NN	N80_C.
NS0_C._N	N.0_C06	N.N_C06	NS0	6N	NN.0	N6	
NS0__.6N	N.0_C06	6.6_C06	_60	N6	NN.6	N	
NS0_CN0	N.0_C06	N.6_C06	NS0	N0	06.0	0N	N80_C.
NS0_CS0	N.0_C06	0.N_C06	NN0	6N	N0.0	NN	
NS0_CNN	N.0_C06	N.N_C06	N_0	_0	N6.N	N5	
NS0_C.NN	N.0_C06	0.0_C06	N_0	06	NN._	S	
NS0_C.NN	N.0_C06	0.N_C06	NN0	NN	NN.0	0	
NS0_C.NN	N.0_C06	0.6_C06	NN0	N6	N0.0	0	
NS0_C.0N	N.0_C06	N.6_C06	N_0	N6	N5.N	N0	

Table 2. Characterization of stable NS0:19.7E cell lines

Cell Line	Seed Density	Final Density	Final Viability	19.7E (µg/ml)	Doubling Time (hours)	Productivity (pg/cell/day)	
NS0_1.18	N.0_105	5.0_105	NN0	N5	1N.8	S	
NS0_S.1N	N.0_105	N.8_105	NN0	1S1	N5.5	N5	N80_1.
NS0_5.S1	N.0_105	N.0_105	NN0	NN	N1.S	S	
NS0_N.5	N.0_105	S.N_105	NN0	10	NN.0	N	
NS0_S.8N	N.0_105	1.1_105	NS0	N5	NN.S	N1	N80_1.
NS0_15.N1	N.0_105	N.8_105	N80	N0	N5.5	8	
NS0_15.SN	N.0_105	N.8_105	NS0	55	N5.5	1S	N80_1.
NS0_18.5N	N.0_105	S.N_105	NN0	50	NN.0	N	
NS0_1N.NN	N.0_105	N.N_105	NS0	N5	N5.N	5	
NS0_15.5N	N.0_105	S.S_105	NN0	N5	N5.5	N	
NS0_11.N5	N.0_105	N.8_105	NN0	S1	N5.5	S	
NS0_11.1N	N.0_105	N.5_105	NN0	S5	N5.N	8	

MILESTONE 4: Field testing of commercial Lassa fever ELISA and lateral flow point-of-care diagnostics in West Africa.

4A. Determine preclinical feasibility of the commercial grade antigen-capture and antibody-capture LASV ELISA using confirmed Lassa fever clinical samples and control samples to achieve benchmarks required for FDA approval.

We are conducting a formalized study of the commercial grade antigen-capture and antibody-capture LASV ELISA and the Lassa LFI. These assays are being compared to each other and to LASV PCR. Early (intermin) analysis of results is included in Figures 8 and 9.

Pivotal study interim analysis (very early) :
Ag ELISA vs. PCR (acute Lassa fever)*

Antigen-capture ELISA

PCR	Negative	Positive	Total
Negative	132	1	133
Positive	1	12	13¥
Total	133	13	146

Sensitivity = 93% (68-100%) PPV = 93% (68-100%)
Specificity = 99% (96-100%) NPV = 99% (96-100%)

*three samples were excluded from the analysis with indeterminant multiple bands or conflicting results on PCR pending sequence analysis.
¥ Enrollment target = 100

Figure 8. Ag-capture assay interim analysis of pivotal trial – early results. Samples with arraws appear to be either false posiutive or negative on the PCR.

Pivotal study interim analysis (very early) :
LFI vs. PCR (acute Lassa fever)*

Lateral Flow

PCR	Negative	Positive	Total
Negative	134	0	134
Positive	2	13	15¥
Total	136	13	149

Sensitivity = 87% (60-98%) PPV = 100% (75-100%)
Specificity = 100% (97-100%) NPV = 97% (95-100%)

*three samples were excluded from the analysis with indeterminant multiple bands or conflicting results on PCR pending sequence analysis.
¥ Enrollment target = 100

Figure 9. LFI interim analysis of pivotal trial – early results

4B. Evaluate and compare LASV ELISA, lateral flow point-of-care diagnostics and polymerase chain reaction assay performance using clinical isolates from diverse regions in the Lassa fever endemic range and areas of West Africa that have high and low prevalences of Lassa.

In 2012, the Nigerian Federal Ministry of Health reported an increased incidence of suspected LF cases. As of July 2012, a total of 933 suspected LF cases, 147 laboratory-confirmed and 93 deaths were reported from 41 local government agencies in 23 States. Phylogenetic analysis shows that LASV strains currently circulating in Nigeria cluster within lineages II and III. These are distinct from lineage I strains represented by the prototype Nigerian Pinneo strain identified in the first characterized LF case in 1969, and from lineage IV strains including the prototype Sierra Leone Josiah strain and other strains circulating in the Mano River Union (MRU) countries of Sierra Leone, Guinea and Liberia.

This study was performed in April 2012 on two panels of samples from recent Nigerian patients suspected of LF. Sample panel A was comprised of plasma samples that tested positive by RT-PCR at ISTH and were subsequently shipped to LUTH. Of the 20 samples in panel A, ten samples were positive and one was weakly positive (±) by the same RT-PCR assay upon retesting at LUTH. These same 20 samples were again re-tested with only six samples positive and one weakly positive by RT-PCR. The fact that fewer samples remained positive on each successive retest indicates that LASV RNA degrades over a short period of time (a few weeks to a few months). At the final time-point, five samples were also positive by Ag-capture ELISA. This included four samples testing positive by RT-PCR at the same time, as well as one sample only detected at the first two time-points. All samples with the exception of one were negative on the LF LFI. Sample degradation may affect the ability to detect NP by LFI.

Sample panel B was comprised of 52 Nigerian patients suspected of LF referred directly to LUTH. Of the 52 samples in Panel B, eight samples originally tested at LUTH were positive or weakly positive. Upon retesting by VHFC, only one of the eight samples was positive by RT-PCR; this was also the only one of the eight samples that was Ag-capture ELISA positive. The results with Panel B confirm the possibility of sample degradation over short periods of time. Of note, five samples negative on both the initial RT-PCR at LUTH and the retest were positive by Ag-capture ELISA. Studies in Sierra Leone indicate that the Ag-capture ELISA produces very few false-positive reactions, and to data cross-reactivity with unrelated viruses has not been observed, suggesting that current RT-PCR primers may not amplify some currently circulating LASV strains in Nigeria. This study points to a critical need to increase point-of-care diagnostics and surveillance of active Lassa fever in Nigeria using alternate testing platforms that keep pace with rapidly emerging viral strain diversity. The increasing failures of RT-PCR diagnosis of LF patients at each successive time-point highlights the need for contemporaneous, point-of-care, testing of potential LF cases. Samples degraded rapidly for both PCR and immunoassays. A point-of-care LF diagnostic similar to that currently used in Sierra Leone would be useful in settings throughout Nigeria, including resource-limited regions.

4C. Develop and perform preclinical testing of algorithms for optimal diagnosis of Lassa fever in bioterrorism and public health scenarios.

Our results indicate that Ag-capture ELISA employing affinity purified goat polyclonal antibodies is useful across the endemic range of LASV in West Africa, and is a promising avenue for the development of point-of-care diagnostics in Nigeria. RT-PCR is the most sensitive LASV diagnostic tool given its amplification capabilities and is an important resource. However, it is highly susceptible to emerging strain diversity that cannot be easily identified until partial or full

genome sequencing is obtained from suspected samples, and is difficult to implement at remote sites. An immunodiagnostic employing high affinity polyclonal antibodies may be less impacted by non-conservative mutations in the viral genome, and could result in prolonged use of this platform in the diagnosis of LF. The identification of samples that were detectable by Ag-capture ELISA, but not by the current RT-PCR platform, suggests this may be the case. The LFI based on the LASV Josiah strain, on the other hand, performed less than optimally at least in part due to sample degradation and may have to be modified for use in Nigeria.

C. SIGNIFICANCE

Viral hemorrhagic fevers (VHFs) are serious, often fatal, illnesses characterized by high fever, damage to the vascular system and multi-organ failure. Because of their high case fatality rates, ability to spread easily by human-human contact and potential for deliberate release, many VHF agents are classified in Biosafety Level 4 and NIAID Biodefense category A. The public health impact of Lassa fever in endemic areas is immense (Birmingham and Kenyon, 2001). Although the current lack of widely available diagnostic tests has precluded accurate surveillance, it has been estimated that there are up to 300,000 cases of Lassa per year in West Africa and 5,000 deaths. In some parts of Sierra Leone 10-15% of all patients admitted to hospitals have Lassa fever, which places a tremendous burden on an already fragile health care infrastructure. Case fatality rates for Lassa fever are typically 15% to 20%, although in epidemics overall mortality can be much higher (>45%). The mortality rate for women in the last month of pregnancy is always high, ~90%, and LASV infection causes high rates of fetal death at all stages of gestation. Mortality rates for Lassa fever appear to be higher in non-Africans, which is of concern because Lassa fever is the most commonly exported VHF. The proposed project will fill a critical biodefense and public health gap by developing modern, commercially-available diagnostic assays for Lassa fever. The potential use of LASV as a biological weapon directed against civilian or military targets necessitates the commercial development of effective diagnostics. The dependence of effective treatment on early diagnosis also provides a strong rationale for improving LASV diagnostics. Furthermore, no LASV vaccine is currently available. Effective immunodiagnostic assays are absolutely essential for development and field testing of potential Lassa fever vaccines.

D. PLANS

Milestone 1

Plans are unchanged from originally proposed. The major goal remains to complete development of commercial Lassa fever ELISA.

Milestone 2

Plans are unchanged from originally proposed. The major goal remains to develop commercial Lassa fever LFI assay with sensitivities and specificities that are similar to LF ELISA.

Milestone 3

Plans are unchanged from originally proposed. The major goal remains to determination which LF analytes are needed and how to best produce them with reasonable cost and scale.

Milestone 4

Plans are unchanged from originally proposed. The major goal remains to perform field testing of commercial Lassa fever ELISA and lateral flow point-of-care diagnostics in West Africa.
Immediate goals are to:

- Define seroprevalence of LASV in Kenema District, Sierra Leone and Irrua, Nigeria as well as in nonendemic regions of Sierra Leone and Nigeria.

- Efforts are currently underway by the VHFC to develop an LFI platform suitable for use in Nigeria, namely for identification of circulating LASV lineages II and III.

E. PUBLICATIONS for project year

Gire SK, Stremlau M, Andersen KG, Schaffner SF, Bjornson Z, McCormick J, Lander ES, Garry RF, Happi C, Sabeti PC. (2012). Viral Hemorrhagic Fevers: Emerging Disease or Emerging Diagnoses? *Science* **338**, 750-752.

F. Project-generated resources

none

Program Director/Principal Investigator (Last, first, middle): Garry, Robert F.

GRANT NUMBER
1U01AI082119-05

CHECKLIST-TULANE

1. PROGRAM INCOME ***(See Instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)
	none	

2. ASSURANCES/CERTIFICATIONS ***(See Instructions.)***
In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III of the PHS 398, and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after the Progress Report (Form Page 5).

3. FACILITIES AND ADMINSTRATIVE (F&A) COSTS
Indicate the applicant organization's most recent F&A cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office.

F&A costs will ***not*** be paid on construction grants, grants to Federal organizations, grants to individuals, and conference grants. Follow any additional instructions provided for Research Career Awards, Institutional National Research Service Awards, Small Business Innovation Research/Small Business Technology Transfer Grants, foreign grants, and specialized grant applications.

☒ DHHS Agreement dated: 04-13-12 ☐ No Facilities and Administrative Costs Requested.

☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION*

Entire proposed budget period: Amount of base $ 358,510 x Rate applied 0.49 % = F&A costs $ 175,670

Add to total direct costs from Form Page 2 and enter new total on Face Page, Item 8b.

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.)*:

Program Director/Principal Investigator (Last, first, middle): Garry, Robert F.

GRANT NUMBER
1U01AI082119-05

CHECKLIST - AIT

1. PROGRAM INCOME ***(See Instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)
	none	

2. ASSURANCES/CERTIFICATIONS ***(See instructions.)***
In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III of the PHS 398, and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after the Progress Report (Form Page 5).

3. FACILITIES AND ADMINSTRATIVE (F&A) COSTS
Indicate the applicant organization's most recent F&A cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office.

F&A costs will ***not*** be paid on construction grants, grants to Federal organizations, grants to individuals, and conference grants. Follow any additional instructions provided for Research Career Awards, Institutional National Research Service Awards, Small Business Innovation Research/Small Business Technology Transfer Grants, foreign grants, and specialized grant applications.

☐ DHHS Agreement dated: _____ ☒ No Facilities and Administrative Costs Requested.

☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION*

Entire proposed budget period: Amount of base $ _____ x Rate applied _____ % = F&A costs $ _____

Add to total direct costs from Form Page 2 and enter new total on Face Page, Item 8b.

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.)*:

Program Director/Principal Investigator (Last, first, middle): Garry, Robert F.

GRANT NUMBER
1U01AI082119-05

CHECKLIST - Corgenix

1. PROGRAM INCOME ***(See instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)
	none	

2. ASSURANCES/CERTIFICATIONS ***(See instructions.)***
In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III of the PHS 398, and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after the Progress Report (Form Page 5).

3. FACILITIES AND ADMINSTRATIVE (F&A) COSTS
Indicate the applicant organization's most recent F&A cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office.

F&A costs will ***not*** be paid on construction grants, grants to Federal organizations, grants to individuals, and conference grants. Follow any additional instructions provided for Research Career Awards, Institutional National Research Service Awards, Small Business Innovation Research/Small Business Technology Transfer Grants, foreign grants, and specialized grant applications.

☐ DHHS Agreement dated: _____ ☒ No Facilities and Administrative Costs Requested.

☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION*

Entire proposed budget period: Amount of base $ _____ x Rate applied _____ % = F&A costs $ _____

Add to total direct costs from Form Page 2 and enter new total on Face Page, Item 8b.

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.):*

Program Director/Principal Investigator (Last, first, middle): Garry, Robert F.

GRANT NUMBER
1U01AI082119-05

CHECKLIST-Vybion

1. PROGRAM INCOME ***(See instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)
	none	

2. ASSURANCES/CERTIFICATIONS ***(See instructions.)***
In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III of the PHS 398, and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after the Progress Report (Form Page 5).

3. FACILITIES AND ADMINSTRATIVE (F&A) COSTS
Indicate the applicant organization's most recent F&A cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office.

F&A costs will ***not*** be paid on construction grants, grants to Federal organizations, grants to individuals, and conference grants. Follow any additional instructions provided for Research Career Awards, Institutional National Research Service Awards, Small Business Innovation Research/Small Business Technology Transfer Grants, foreign grants, and specialized grant applications.

☒ DHHS Agreement dated: 05-08-07 ☐ No Facilities and Administrative Costs Requested.

☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION*

Entire proposed budget period: Amount of base $ 117,087 x Rate applied 100% % = F&A costs $ 117,087

Add to total direct costs from Form Page 2 and enter new total on Face Page, Item 8b.

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.)*:

ALL PERSONNEL REPORT

Place this form at the end of the signed original copy of the application. Do not duplicate.

GRANT NUMBER
1U01AI082119-05

Always list the PD/PI(s). In addition, list all other personnel who participated in the project during the current budget period for at least one person month or more, regardless of the source of compensation (a person month equals approximately 160 hours or 8.3% of annualized effort). Use the following abbreviated categories for describing Role on Project:

- **PD/PI**
- **Co-Investigator**
- **Faculty Collaborator**
- **Staff Scientist (doctoral level)**
- **Postdoc (Postdoctoral Scholar, Fellow, or Other Postdoctoral Position)**
- **Grad Rsch Asst (Graduate Research Assistant)**
- **Undergrad Rsch Asst (Undergraduate Research Assistant)**
- **Rsch Asst (Research Assistant/Coordinator)**
- **Technician**
- **Consultant**
- **Biostatistician**
- **Other (Specify)**

If personnel are supported by a Reentry or Diversity Supplement or American Recovery and Reinvestment Act (ARRA) funding, please indicate such after the Role on Project, using the following abbreviations: RS - Reentry Supplement; DS - Diversity Supplement; AF - General ARRA Supplement; ASE - ARRA Summer Experience funding.

Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
eRA Commons User Name	Robert F. Garry	BS, PhD	Personal Info	PI	Personal Info	EFFORT		
	John Scheiffelin	BS, MD		Investigator				
	Allyson M. Haislip	MS		Rsch asst				
	Lina Moses	PhD		Other: Field Coordinator				
	Chris Bishop	BS		Other: Project Manager				
	Personal Info	BS		Grad Rsch Asst				
		BS		Grad Rsch Asst				
	Russell B. Wilson	PhD		Director, subcontract				
	Peter C. Kulakosky	PhD		Co-Investigator				
	Luis M. Branco	PhD	Personal Info	Co-Investigator	Personal Info	EFFORT		
	Stefanie McCormick	BS		Technician				
	Tamera Nelson	BS		Technician				
	Milele Ramsey	BS		Technician				

ALL PERSONNEL REPORT- CONTINUED

Place this form at the end of the signed original copy of the application. Do not duplicate.

GRANT NUMBER
1U01AI082119-05

Always list the PD/PI(s). In addition, list all other personnel who participated in the project during the current budget period for at least one person month or more, regardless of the source of compensation (a person month equals approximately 160 hours or 8.3% of annualized effort). Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID	Name	Degree(s)	SSN (last 4 digits)	Role on Project (e.g. PD/PI, Res. Assoc.)	DoB (MM /YY)	Cal	Acad	Summer
eRA Commons User Name	Kelly Pitts	PhD	Personal Info	Director, subcontract	Personal Info	EFFORT		
	Dorin Oottamasathien	BS		Other: R&D Staff: Production				
	Matt Boisen	BS		Other: R&D Staff: Development				
	Daniel F. Simpson	BS		Other: R&D Staff: Regulatory Affairs				
	Bethany Belote	BS		Other: R&D Staff: Quality Control				
	Abby Jones	BS		Other: R&D Staff: Manufacturing				
eRA Commons User Name	Lee A. Henderson	BS, PhD		Director, subcontract				
	Hui Zhu	MS		Other: R&D Staff: Fermentation :				

Grant Number: 1U19AI115589-01
FAIN: U19AI115589

Principal Investigator(s):
Robert F Garry, PHD

Project Title: International Collaboration in Infectious Disease Research on Lassa fever

Ms. Kozar, Kathleen M
Director
1430 Tulane Avenue, Ep-15
New Orleans, LA 701122613

Award e-mailed to: elecnotf@tulane.edu

Period Of Performance:
Budget Period: 02/12/2015 – 01/31/2016
Project Period: 02/12/2015 – 01/31/2020

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $670,528 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to TULANE UNIVERSITY OF LOUISIANA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 31 USC 6305 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number U19AI115589. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Tina M. Carlisle
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 1U19AI115589-01

Award Calculation (U.S. Dollars)

Salaries and Wages	$103,751
Fringe Benefits	$21,863
Equipment	$49,727
Supplies	$79,064
Travel Costs	$71,500
Other Costs	$10,600
Consortium/Contractual Cost	$176,575
Federal Direct Costs	$513,080
Federal F&A Costs	$157,448
Approved Budget	$670,528
Total Amount of Federal Funds Obligated (Federal Share)	$670,528
TOTAL FEDERAL AWARD AMOUNT	$670,528
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$670,528

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$670,528	$670,528
2	$670,315	$670,315
3	$675,365	$675,365
4	$675,365	$675,365
5	$668,295	$668,295

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy, Immunology and Transplantation Research
CFDA Number:	93.855
EIN:	1720423889A1
Document Number:	UAI115589A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2015

IC	CAN	2015	2016	2017	2018	2019
AI	8472315	$670,528	$670,315	$675,365	$675,365	$668,295

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M32B B / **OC**: 414L / **Released**: eRA Commons User Name 02/05/2015
Award Processed: 01/15/2015 11:58:50 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1U19AI115589-01

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1U19AI115589-01

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.

c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

Carry over of an unobligated balance into the next budget period requires Grants Management Officer prior approval.

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) U19AI115589. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 1U19AI115589-01

The budget period anniversary start date for future year(s) will be **February** 1.
****

In addition to the PI, any absence, replacement, or substantial reduction in effort of the following individual(s) below, requires the written prior approval of the National Institutes of Health awarding component.

Dr. Jeffrey Schaffer, Co-PI, Project 1 Leader

Dr. John Schieffelin, Co-PI, Project 2 Leader
Dr. Donald Grant, Co-PI, Deputy Director of KGH Viral Hemorrhagic Fever Program
****

This award includes funds for subcontract/consortium activity with Kenema General Hospital in SIERRA LEONE and is budgeted as follows:

	-Yr 1	-Yr 2	-Yr 3	-Yr 4	-Yr 5
Total Direct Costs	$163,495	$181,495	$181,495	$181,495	$181495
F&A Costs @ 8%(MTDC)	$13,080	$14,520	$14,520	$14,520	$14,520
TOTAL COSTS					
$176,575	$196,015	$196,015	$196,015	$196,015	

Consortiums are to be established and administered as described in the NIH Grants Policy Statement. This written agreement with the consortium must address the negotiated arrangements for meeting the scientific, administrative, financial, and reporting requirements for this grant.
****

This award is issued as a Cooperative Agreement, a financial assistance mechanism in which substantial NIH scientific and/or programmatic involvement is anticipated in the performance of the activity. This award is subject to the Terms and Conditions of Award as set forth in Section VI: Award Administrative Information of RFA AI-14-001, “International Collaborations in Infectious Diseases Research (U01),” release date 11/27/2013, which are hereby incorporated by reference as special terms and conditions of this award.

This RFA may be accessed at: http://grants.nih.gov/grants/guide/index.html
****

Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at http://www.selectagents.gov/Regulations.html) must complete registration with CDC (or APHIS, depending on the agent) before using NIH funds for any work directly involving the Select Agent at the US institution. No funds can be used for research involving Select Agents if the final registration certificate is denied. Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS.

Before using NIH funds for any work directly involving the Select Agents at the foreign institution, the US awardee must provide information from the foreign institution satisfactory to the NIAID that processes and requirements comparable to those described in 42 CFR 73 for US institutions are in place and will be administered on behalf of all Select Agent work sponsored by NIH funds.

The US awardee must work with the foreign institution to ensure:

- That they understand the NIAID Select Agent Policy and requirements,
- That they are willing and able to allow the NIAID representative to enter and review the laboratories or facilities where Select Agent research is (or will be) conducted and the area(s) where NIAID funded select agents and toxins are stored,
- That they are willing and able to allow site reviews every three years after the initial review
- And that, during the visit, they are willing to address the following key elements appropriate for their institution: safety, security, incident response plan, training, procedures for personnel security risk assessment ensuring that only approved/appropriate individuals have access to the Select Agents, and any applicable laws, regulations and policies equivalent to 42 CFR 73.

If this work will not, in fact, involve Select Agents subject to the provisions of the US Select Agents regulations (e.g. excluded strains), and the US awardee provides documentation satisfactory to the NIAID that the work does not now nor will it in the future (i.e. throughout the life of the award) involve Select Agents at the foreign institution, no further action will be necessary.

Prior to conducting a restricted experiment with a Select Agent or Toxin at the foreign institution, the US awardees must request and receive approval from NIAID.
****

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Tina M. Carlisle
Email: carlislt@niaid.nih.gov **Phone**: 240-669-2947 **Fax**: 301-493-0597

Program Official: Patricia M. Repik

Email: prepik@niaid.nih.gov **Phone**: 301-496-7453 **Fax**: 301-480-1594

SPREADSHEET SUMMARY
GRANT NUMBER: 1U19AI115589-01

INSTITUTION: TULANE UNIVERSITY OF LOUISIANA

Budget	Year 1	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	$103,751	$103,751	$103,751	$103,751	$103,751
Fringe Benefits	$21,863	$21,863	$21,863	$21,863	$21,863
Equipment	$49,727	$10,000			$14,000
Supplies	$79,064	$98,191	$106,191	$106,191	$94,191
Travel Costs	$71,500	$71,500	$71,500	$71,500	$71,500
Other Costs	$10,600	$13,200	$15,200	$15,200	$13,200
Consortium/Contractual Cost	$176,575	$196,015	$196,015	$196,015	$196,015
TOTAL FEDERAL DC	$513,080	$514,520	$514,520	$514,520	$514,520
TOTAL FEDERAL F&A	$157,448	$155,795	$160,845	$160,845	$153,775
TOTAL COST	$670,528	$670,315	$675,365	$675,365	$668,295

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	50.5%	50.5%	50.5%	50.5%	50.5%
F&A Cost Base 1	$311,778	$308,505	$318,505	$318,505	$304,505
F&A Costs 1	$157,448	$155,795	$160,845	$160,845	$153,775

PI: **Garry, Robert F**	Title: International Collaboration in Infectious Disease Research on Lassa fever	
Received: 03/07/2014	FOA: AI14-002	Council: 10/2014
Competition ID: FORMS-C	FOA Title: INTERNATIONAL COLLABORATIONS IN INFECTIOUS DISEASES RESEARCH (U19)	
1 U19 AI115589-01	Dual:	Accession Number: 3678445
IPF: 8424601	Organization: TULANE UNIVERSITY OF LOUISIANA	
Former Number:	Department:	
IRG/SRG: ZAI1 AWA-M (S2)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 500,000 Year 2: 500,000 Year 3: 500,000 Year 4: 500,000 Year 5: 500,000	Animals: Y Humans: Y Clinical Trial: N Current HS Code Evaluative Info HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Robert Garry	TULANE UNIVERSITY	PD/PI
Sheik Khan M.D.	Kenema Givernment Hspital	Co-PD/PI
John Schieffelin	Tulane University	Co-PD/PI
Jeffrey Shaffer	Tulane University	Co-PD/PI

Appendices

Overall_Multiple_P

Additions for Review

Supplemental Material	GarryU19_Letter_Bio_Grant.pdf

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*
❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED 2014-03-07

Application Identifier

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS*:** 0537858120000

Legal Name*: TULANE UNIVERSITY
Department:
Division:
Street1*: TULANE UNIVERSITY
Street2: 6823 ST. CHARLES AVE
City*: NEW ORLEANS
County:
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 701180000

Person to be contacted on matters involving this application
Prefix: Ms. First Name*: Kathleen Middle Name: M Last Name*: Kozar Suffix:
Position/Title: Director
Street1*: 1430 Tulane Avenue, Ep-15
Street2:
City*: New Orleans
County: Orleans
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 701122613
Phone Number*: 5049885613 Fax Number: 5049881748 Email: elecnot@tulane.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 720423889

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*
● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
International Collaboration in Infectious Disease Research on Lassa fever

12. PROPOSED PROJECT
Start Date* 01/01/2015
Ending Date* 12/31/2019

13. CONGRESSIONAL DISTRICTS OF APPLICANT
LA-002

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Robert Middle Name: F Last Name*: Garry Suffix:
Position/Title:
Organization Name*: TULANE UNIVERSITY
Department:
Division:
Street1*: TULANE UNIVERSITY
Street2: DEPARTMENT OF MICROBIOL/IMMUNOLOGY
City*: NEW ORLEANS
County:
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 701122613
Phone Number*: (504) 988-2027 Fax Number: (504) 988-1994 Email*: rfgarry@tulane.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$3,276,083.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$3,276,083.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Ms. First Name*: Kathleen Middle Name: M. Last Name*: Kozar Suffix:
Position/Title*: Director
Organization Name*: Tulane University
Department: Sponsored Projects Admin.
Division:
Street1*: 1430 Tulane Avenue, Ep-15
Street2:
City*: New Orleans
County: Orleans
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 701122632
Phone Number*: 5049985613 Fax Number: 5049881748 Email*: kkozar@tulane.edu

Signature of Authorized Representative*
Tami Jenniskens

Date Signed*
03/07/2014

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific Table Of Contents

Component Summary

Components	Component Project Title	Contact PD/PI Name or Project Lead Name
Overall	International Collaboration in Infectious Disease Research on Lassa fever	Garry, Robert F
Admin-Core-001 (657)	Lassa ICIDR - Administrative Core	Garry, Robert F
Project-001 (819)	Project 1: Evaluation of second generation Lassa fever immunoassays as point-of-care diagnostics and surveillance tools for Lassa fever.	Shaffer, Jeffrey George
Project-002 (839)	Project 2. Expansion of clinical research capacity at Kenema Government Hospital	Schieffelin, John Scribner

Project/Performance
Site Location(s) Summary

Applicant Organization	City	State/Province	Country
TULANE UNIVERSITY	NEW ORLEANS	LA	UNITED STATES

Organization Name	City	State/Province	Country	Component
Kenema Government Hospital	Kenema		SIERRA LEONE	Admin-Core-001 (657)
Kenema Government Hospital	Kenema		SIERRA LEONE	Overall
Kenema Government Hospital	Kenema		SIERRA LEONE	Project-001 (819)
Kenema Government Hospital	Kenema		SIERRA LEONE	Project-002 (839)
TULANE UNIVERSITY	NEW ORLEANS	LA	UNITED STATES	Admin-Core-001 (657)
Tulane University	New Orleans	LA	UNITED STATES	Overall
TULANE UNIVERSITY	NEW ORLEANS	LA	UNITED STATES	Project-001 (819)
TULANE UNIVERSITY	NEW ORLEANS	LA	UNITED STATES	Project-002 (839)

Human Subjects
Clinical Trial
Human Embryonic Stem Cells
Vertebrate Animals
Summary

Components	Human Subjects	Clinical Trial	HESC Involved	Vertebrate Animals
Overall	Y	N	N	Y
Admin-Core-001 (657)	N	N	N	N
Project-001 (819)	Y	N	N	Y
Project-002 (839)	Y	N	N	N

Composite Application Budget Summary

Categories	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Salary, Wages and Fringe Benefits	125,614	125,614	125,614	125,614	125,614	628,070
Equipment	49,727	10,000	0	0	14,000	73,727
Travel	71,500	71,500	71,500	71,500	71,500	357,500
Participant/Trainee Support Costs	8,000	8,000	10,000	10,000	8,000	44,000
Other Direct Costs (excluding Consortium)	81,664	103,391	111,391	111,391	99,391	507,228
Consortium Costs	163,495	181,495	181,495	181,495	181,495	889,475
Direct Costs	500,000	500,000	500,000	500,000	500,000	2,500,000
Indirect Costs	144,823	155,795	160,845	160,845	153,775	776,083
Total Direct and Indirect Costs	644,823	655,795	660,845	660,845	653,775	**3,276,083**

Total Direct Costs less Consortium F&A

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	500,000	500,000	500,000	500,000	500,000	**2,500,000**

Component Budget Summary

Components	Categories	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Admin-Core-001 (657)	Salary, Wages and Fringe Benefits	27,877	27,877	27,877	27,877	27,877	139,385
	Equipment	0	0	0	0	0	0
	Travel	6,500	6,500	6,500	6,500	6,500	32,500
	Participant/Trainee Support Costs	0	0	0	0	0	0
	Other Direct Costs (excluding Consortium)	623	623	623	623	623	3,115
	Consortium Costs	15,000	15,000	15,000	15,000	15,000	75,000
	Direct Costs	50,000	50,000	50,000	50,000	50,000	250,000
	Indirect Costs	17,675	17,675	17,675	17,675	17,675	88,375
TOTALS	Total Direct and Indirect Costs	67,675	67,675	67,675	67,675	67,675	**338,375**
Project-001 (819)	Salary, Wages and Fringe Benefits	49,042	49,042	49,042	49,042	49,042	245,210
	Equipment	45,727	10,000	0	0	10,000	65,727
	Travel	25,000	25,000	25,000	25,000	25,000	125,000
	Participant/Trainee Support Costs	8,000	8,000	10,000	10,000	8,000	44,000
	Other Direct Costs (excluding Consortium)	35,736	53,463	61,463	61,463	53,463	265,588
	Consortium Costs	76,495	94,495	94,495	94,495	94,495	454,475
	Direct Costs	240,000	240,000	240,000	240,000	240,000	1,200,000
	Indirect Costs	59,478	68,430	73,480	73,480	68,430	343,298
TOTALS	Total Direct and Indirect Costs	299,478	308,430	313,480	313,480	308,430	**1,543,298**

Project-002 (839)	Salary, Wages and Fringe Benefits	48,695	48,695	48,695	48,695	48,695	243,475
	Equipment	4,000	0	0	0	4,000	8,000
	Travel	40,000	40,000	40,000	40,000	40,000	200,000
	Participant/Trainee Support Costs	0	0	0	0	0	0
	Other Direct Costs (excluding Consortium)	45,305	49,305	49,305	49,305	45,305	238,525
	Consortium Costs	72,000	72,000	72,000	72,000	72,000	360,000
	Direct Costs	210,000	210,000	210,000	210,000	210,000	1,050,000
	Indirect Costs	67,670	69,690	69,690	69,690	67,670	344,410
TOTALS	Total Direct and Indirect Costs	277,670	279,690	279,690	279,690	277,670	**1,394,410**
TOTALS		**644,823**	**655,795**	**660,845**	**660,845**	**653,775**	**3,276,083**

Categories Budget Summary

Categories	Components	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
R&R Budget - Senior/Key Person Funds Requested	Admin-Core-001 (657)	21,435	21,435	21,435	21,435	21,435	107,175
	Project-001 (819)	49,042	49,042	49,042	49,042	49,042	245,210
	Project-002 (839)	48,695	48,695	48,695	48,695	48,695	243,475
TOTALS		119,172	119,172	119,172	119,172	119,172	**595,860**
R&R Budget - Other Personnel Funds Requested	Admin-Core-001 (657)	6,442	6,442	6,442	6,442	6,442	32,210
	Project-001 (819)	0	0	0	0	0	0
	Project-002 (839)	0	0	0	0	0	0
TOTALS		6,442	6,442	6,442	6,442	6,442	**32,210**
R&R Budget - Section A & B. Total Salary, Wages and Fringe Benefits (A+B)	Admin-Core-001 (657)	27,877	27,877	27,877	27,877	27,877	139,385
	Project-001 (819)	49,042	49,042	49,042	49,042	49,042	245,210
	Project-002 (839)	48,695	48,695	48,695	48,695	48,695	243,475
TOTALS		125,614	125,614	125,614	125,614	125,614	**628,070**
R&R Budget - Section C. Total Equipment	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	45,727	10,000	0	0	10,000	65,727
	Project-002 (839)	4,000	0	0	0	4,000	8,000
TOTALS		49,727	10,000	0	0	14,000	**73,727**
R&R Budget - Domestic Travel	Admin-Core-001 (657)	2,000	2,000	2,000	2,000	2,000	10,000

	Project-001 (819)	0	0	0	0	0	0
	Project-002 (839)	4,000	4,000	4,000	4,000	4,000	20,000
TOTALS		6,000	6,000	6,000	6,000	6,000	**30,000**
R&R Budget - Foreign Travel	Admin-Core-001 (657)	4,500	4,500	4,500	4,500	4,500	22,500
	Project-001 (819)	25,000	25,000	25,000	25,000	25,000	125,000
	Project-002 (839)	36,000	36,000	36,000	36,000	36,000	180,000
TOTALS		65,500	65,500	65,500	65,500	65,500	**327,500**
R&R Budget - Section D. Total Travel	Admin-Core-001 (657)	6,500	6,500	6,500	6,500	6,500	32,500
	Project-001 (819)	25,000	25,000	25,000	25,000	25,000	125,000
	Project-002 (839)	40,000	40,000	40,000	40,000	40,000	200,000
TOTALS		71,500	71,500	71,500	71,500	71,500	**357,500**
R&R Budget - Tuition/Fees/Health Insurance	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	3,000	3,000	4,000	4,000	3,000	17,000
	Project-002 (839)	0	0	0	0	0	0
TOTALS		3,000	3,000	4,000	4,000	3,000	**17,000**
R&R Budget - Stipends	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	0	0	0	0	0	0
	Project-002 (839)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget - Trainee Travel	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	5,000	5,000	6,000	6,000	5,000	27,000

	Project-002 (839)	0	0	0	0	0	0
TOTALS		5,000	5,000	6,000	6,000	5,000	**27,000**
R&R Budget - Subsistence	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	0	0	0	0	0	0
	Project-002 (839)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget ï¿½ Other Participants/Trainee Support Costs	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	0	0	0	0	0	0
	Project-002 (839)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget ï¿½ Section E. Total Participants/Trainee Support Costs	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	8,000	8,000	10,000	10,000	8,000	44,000
	Project-002 (839)	0	0	0	0	0	0
TOTALS		8,000	8,000	10,000	10,000	8,000	**44,000**
R&R Budget ï¿½ Materials and Supplies	Admin-Core-001 (657)	623	623	623	623	623	3,115
	Project-001 (819)	35,736	50,863	58,863	58,863	50,863	255,188
	Project-002 (839)	42,705	46,705	46,705	46,705	42,705	225,525
TOTALS		79,064	98,191	106,191	106,191	94,191	**483,828**
R&R Budget ï¿½ Publication Costs	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	0	2,600	2,600	2,600	2,600	10,400
	Project-002 (839)	2,600	2,600	2,600	2,600	2,600	13,000

TOTALS		2,600	5,200	5,200	5,200	5,200	**23,400**
R&R Budget ï¿½ Consultant Services	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	0	0	0	0	0	0
	Project-002 (839)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget ï¿½ ADP/Computer Services	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	0	0	0	0	0	0
	Project-002 (839)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget ï¿½ Subawards/Consortium/Contractual Costs	Admin-Core-001 (657)	15,000	15,000	15,000	15,000	15,000	75,000
	Project-001 (819)	76,495	94,495	94,495	94,495	94,495	454,475
	Project-002 (839)	72,000	72,000	72,000	72,000	72,000	360,000
TOTALS		163,495	181,495	181,495	181,495	181,495	**889,475**
R&R Budget ï¿½ Equipment or Facility Rental User Fees	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	0	0	0	0	0	0
	Project-002 (839)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget ï¿½ Alterations and Renovations	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	0	0	0	0	0	0
	Project-002 (839)	0	0	0	0	0	0

TOTALS		0	0	0	0	0	**0**
R&R Budget ï¿½ Other Direct Cost 1	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	0	0	0	0	0	0
	Project-002 (839)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget ï¿½ Other Direct Cost 2	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	0	0	0	0	0	0
	Project-002 (839)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget ï¿½ Other Direct Cost 3	Admin-Core-001 (657)	0	0	0	0	0	0
	Project-001 (819)	0	0	0	0	0	0
	Project-002 (839)	0	0	0	0	0	0
TOTALS		0	0	0	0	0	**0**
R&R Budget ï¿½ Section F. Total Other Direct Cost	Admin-Core-001 (657)	15,623	15,623	15,623	15,623	15,623	78,115
	Project-001 (819)	112,231	147,958	155,958	155,958	147,958	720,063
	Project-002 (839)	117,305	121,305	121,305	121,305	117,305	598,525
TOTALS		245,159	284,886	292,886	292,886	280,886	**1,396,703**
R&R Budget ï¿½ Section G. Total Direct Cost (A thru F)	Admin-Core-001 (657)	50,000	50,000	50,000	50,000	50,000	250,000
	Project-001 (819)	240,000	240,000	240,000	240,000	240,000	1,200,000
	Project-002 (839)	210,000	210,000	210,000	210,000	210,000	1,050,000
TOTALS		500,000	500,000	500,000	500,000	500,000	**2,500,000**

R&R Budget ï¿½ Section H. Indirect Costs	Admin-Core-001 (657)	17,675	17,675	17,675	17,675	17,675	88,375
	Project-001 (819)	59,478	68,430	73,480	73,480	68,430	343,298
	Project-002 (839)	67,670	69,690	69,690	69,690	67,670	344,410
TOTALS		144,823	155,795	160,845	160,845	153,775	**776,083**
R&R Budget ï¿½ Section I. Total Direct and Indirect Costs (G +H)	Admin-Core-001 (657)	67,675	67,675	67,675	67,675	67,675	338,375
	Project-001 (819)	299,478	308,430	313,480	313,480	308,430	1,543,298
	Project-002 (839)	277,670	279,690	279,690	279,690	277,670	1,394,410
TOTALS		644,823	655,795	660,845	660,845	653,775	**3,276,083**

Senior/Key Personnel
Summary

Name	Organization	Role on Project	Components
Garry, Robert F	TULANE UNIVERSITY	PD/PI(Contact)	Overall
Branco, Luis M.	Zalgen Labs	Other: External Advisory Group	Admin-Core-001 (657)
Cashman, Kathleen A.	USAMRIID	Other: External Advisory Group	Admin-Core-001 (657)
Garry, Robert F	Tulane University	Co-PD/PI	Admin-Core-001 (657)
Grant, Donald Samuel	Kenema Government Hospital	Co-PD/PI	Project-001 (819)
Khan, Sheik Humarr	Kenema Givernment Hspital	Co-PD/PI	Overall
Khan, Shiek Humarr	Kenema Givernment Hspital	Co-PD/PI	Admin-Core-001 (657)
Khan, Shiek Humarr	Kenema Givernment Hspital	Co-PD/PI	Project-002 (839)
Moses, Lina Michiko	Tulane University	Co-PD/PI	Project-001 (819)
Nunberg, Jack H	University of Montana	Other: External Advisory Group	Admin-Core-001 (657)
Sabeti, Pardis Christine	Harvard University	Other: External Advisory Group	Admin-Core-001 (657)
Saphire, Erica Ollmann	The Scripps Research Institute	Other: External Advisory Group	Admin-Core-001 (657)
Schieffelin, John Scribner	Tulane University	Co-PD/PI	Overall
Schieffelin, John Scribner	Tulane University	Other: Project Lead	Project-002 (839)
Shaffer, Jeffrey George	Tulane University	Co-PD/PI	Overall
Shaffer, Jeffrey George	Tulane University	Co-PD/PI	Project-001 (819)
Shaffer, Jeffrey George	Tulane University	Co-Investigator	Project-002 (839)

BIOGRAPHICAL SKETCH

NAME Robert F. Garry	POSITION TITLE Professor, Microbiology and Immunology
eRA COMMONS USER NAME eRA Commons User Name	Assistant Dean for Graduate Studies in Biomedical Sciences

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Indiana State University, Terre Haute, Indiana	B.S.	1974	Life Sciences
University of Texas, Austin, Texas	Ph.D.	1978	Microbiology
University of Texas, Austin, Texas	Postdoctoral		Virology

A. Personal Statement

Proposal Goal/Role. I will serve as Co-PI of the Lassa ICIDR and Co-PD of the Administrative Core. I will work closely with the Dr. Humarr Khan, a long-time collaborator, to ensure the success of the research and training aspects of the Lassa ICIDR and provide training opportunities for Sierra Leonean staff. I will also work closely with Dr. Khan to ensure effective leadership and oversight of the Program.

Relevant Experiences. I am currently managing a consortium of scientists who are developing countermeasures, including diagnostics, immunotherapeutics and vaccines, against Lassa virus and several other biodefense pathogens (vhfc.org). Our team, in collaboration with Sierra Leonean scientists, has produced arenavirus point-of-care and confirmatory diagnostics based on recombinant proteins and is engaged in a detailed analysis of B cell epitopes in human Lassa fever (LF).

Other Qualifications. I serve in the Tulane University administration as Assistant Dean for Graduate Studies in Biomedical Sciences. I have a long-term commitment to training young scientists. I established (with Dr. David Sander) All the Virology on the World Wide Web (www.virology.net), one of the first Internet index sites for virology, which remains popular today. I am also the founding Editor-in-Chief of *Virology Journal* (Biomed Central; www.virologyj.com), and have served on many NIH review panels.

B. Positions and Honors.

1983 - date Assistant Professor (1983-87); Associate Professor (1987–93); Professor (1993-date) of Microbiology and Immunology at Tulane University School of Medicine at New Orleans, Louisiana 70112.

2012 – date Associate Member: The Broad Institute of Harvard and MIT; Adjunct Professor: Tuskegee University

1995 – date Established (with Dr. David Sander) All the Virology on the World Wide Web.

2006 – date Assistant Dean for Graduate Studies in Biomedical Sciences

2004 Founding Editor-in-Chief: *Virology Journal* (BioMed Central)

1988 - date Member of 6 *Ad hoc* NIH AIDS Study Sections (ARR-3, 1988-90, before charter); Member of NIH AIDS Molecular Biology Study Section (ARR-C, 1990-1996); Member or Chair of over 40 NIAID, NHLBI, MBRS, NCI or HHS Special Study Sections (1996-date), including: Chair, VATID Biodefense Study Section (2002); Chair, SBIR-STTR Biodefense Study Sections (2002-2006), Chair, VATID AIDS vaccine Study Sections (2009, 2010); Co-Chair Regional Centers of Excellence Review panels (2002, 2008); member: OMICS SEP; Member, SBIR-STTR Non-HIV Anti-infectives Study Section (“Bugs and Drugs” 2006-2010); Member, AIDSRRC (2012-date).

C. Selected Peer-reviewed Publications (15 publications selected from >180 peer-reviewed publications)

Most relevant to the current application

Branco, L, Matschiner A, Fair, JN, Goba, A, Ferro, P, Cashman K, Sampey D., Schoepp R, Tesh R, Bausch DB, Garry RF, and Guttieri MC. (2008). Bacterial-based systems for expression and purification of recombinant Lassa virus proteins of immunological relevance. *Virol J.* **5,** 73. PMCID: PMC2435526

Branco LM, Grove JN, Geske FJ, Boisen ML, Muncy IJ, Magliato SA, Henderson LA, Schoepp RJ, Cashman KA, Hensley LE, Garry RF. (2010) Lassa virus-like particles displaying all major immunological determinants as a vaccine candidate for Lassa hemorrhagic fever. *Virol J.* **7,** 279. PMCID: PMC2984592

Branco LM, Grove JN, Boisen ML, Shaffer JG, Goba A, Fullah M, Momoh M, Grant DS, Garry RF. (2011) Emerging trends in Lassa fever: redefining the role of immunoglobulin M and inflammation in diagnosing acute infection. *Virology Journal* 8,478. PMCID: PMC3223505

Gire SK , Stremlau M, Andersen KG, Schaffner SF, Bjornson Z3, Joseph McCormick J, Lander ES, Garry RF, Happi C, Sabeti PC. (2012). Viral Hemorrhagic Fevers: Emerging Disease or Emerging Diagnoses? *Science* **338,** 750-752.

Shaffer JG, Grant D Sand Schieffelin JS … Garry, RF. Lassa fever in post-conflict Sierra Leone. PLoS Neglected Tropical Diseases 2014, in press.

Additional recent publications of importance to the field (in chronological order)

Garry RF, Witte M, Gottlieb, AA., Elvin-Lewis M., Gottlieb M., Witte C., Alexander SS, Cole WR, Drake WL (1988). Documentation of an AIDS virus infection in 1968. *JAMA* **260,** 2085-2087.

Garry RF, and Fermin CD. (1993). Viral burden in AIDS. *Nature* **365,** 301-302, 1993.

Sainz B Jr., Rausch JM, Gallaher WR, Garry RF, Wimley WC. (2005). Identification and characterization of the putative fusion peptide of the severe acute respiratory syndrome-associated coronavirus spike protein. *J Virol*. 79:7195-206. PMCID: PMC1112137.

Illick M.M., Branco, L.M., Fair, J.N., Illick, K.A, Matschiner, A., Schoepp, R., Garry, R.F., Guttieri, M.C. (2008). Uncoupling GP1 and GP2 expression in the Lassa virus glycoprotein complex: implications for GP1 ectodomain shedding. *Virol J* **5,** 161. PMCID: PMC2645378

Khan SH, Goba A, Chu M, Roth C, Healing T, Marx A, Guttieri MC, Ferro P, Imes T, Monagin C, Garry RF, Bausch DG; Mano River Union Lassa Fever Network. (2008). New opportunities for field research on the pathogenesis and treatment of Lassa fever. Antiviral Res. 78, 103-15.

Branco LM, Grove JN, Moses LM, Goba A, Fullah M, Momoh M, Schoepp RJ, Bausch DG, Garry RF. (2010) Shedding of soluble glycoprotein 1 detected during acute Lassa virus infection in human subjects. *Virol J.* **7,** 306. PMCID: PMC2993672

Safronetz D, Lopez JE, Sogoba N, Traore' SF, Raffel SJ, Fischer ER, Ebihara H, Branco L, Garry RF, Schwan TG, Feldmann H. (2010). Detection of Lassa virus, Mali. *Emerg Infect Dis* **16,** 1123-6. PMCID: PMC3321918.

Grove JN, Branco LM, Boisen ML, Muncy IJ, Henderson LA, Schieffellin JS, Robinson JE, Bangura JJ, Fonnie M, Schoepp RJ, Hensley LE, Seisay A, Fair JN, Garry, RF (2011). Capacity building permitting comprehensive monitoring of a severe case of Lassa hemorrhagic fever in Sierra Leone with a positive outcome: Case Report. *Virol J.* **8,** 314. PMCID: PMC3283910

Branco LM, Boisen ML, Andersen KG, Grove JN, Moses LM, Muncy IJ, Henderson LA, Schieffellin JS, Robinson JE, Bangura JJ, Grant DS, Raabe VN, Fonnie M,Sabeti PC, Robert F Garry RF. (2011). Lassa Hemorrhagic Fever in a Late Term Pregnancy from Northern Sierra Leone with a Positive Maternal Outcome: Case Report. *Virol J.* 2011, 8, 404. PMCID: PMC3177908

Lok S-M, Costin JM, Hrobowski YM, Hoffmann AR, Rowe DK, Kukkaro P, Holdaway H, Chipman P, Fontaine KA, Holbrook MA, Garry RF, Kostyuchenko V, Wimley WC, Isern S, Rossmann MG, Michael SF. (2012). Release of dengue virus genome induced by a peptide inhibitor. PlosOne **7,** e50995. PMCID: PMC3511436.

D. Research Support

ACTIVE

1U01AI082119-01 Garry (PI) 06/01/09 - 05/31/14
NIAID
Preclinical development of recombinant antigen diagnostics for Lassa fever
The goal is to complete the preclinical development phase for recombinant IgM-, IgG- and antigen-capture assays and point-of-care lateral flow assays for diagnosis of infection by Lassa virus.
Role: PI

HHSN272200900049C (PI-Robinson) 09/30/09 – 09/29/14
NIAID-DAIT
Roles of protective or pathogenic B cell epitopes in human Lassa fever
The goals are to discover novel B cell epitopes of Lassa virus protein antigens and to elucidate mechanisms of antibody-mediated protection or pathogenesis in a well-characterized cohort of persons exposed to diverse strains of LASV at different stages and with different severities of Lassa fever.
Role: Program Manager responsible for overall scientific and logistical management of the project.

HHSN272201000022C (Co-PIs: Sabeti and Garry) 10/01/10 – 09/30/15
NIAID
Host Genetic Factors in Resistance to Lassa Hemorrhagic Fever
The goals of this Project are to replicate strong signals of natural selection found in Yorubans at genes critical for infection with Lassa virus (LASV) in four additional West African populations and to localize and characterize the key functional mutations.
Role: Director of subcontract.

1R01AI104621-01 04-01-13 - 03-31-18
NIAID
Antibody immunotherapeutics for biodefense against Lassa virus
The objective of this project is to create a safe, well tolerated, and effective drug that will treat or prevent Lassa fever, a severe and often-fatal disease that is a major biowarfare threat. We have isolated human antibodies that block infectivity of the Lassa virus and will use modern molecular biology techniques to generate and modify these antibodies to develop an immunotherapeutic. There is no overlap of this grant with the current application, which will develop broad-spectrum immunotherapeutics against nonLassa Old World arenaviruses and New World arenaviruses.
Role: PI

1R13AI104216-01 12-01-123 – 11/30/2014
NIAID
Opening a New Lassa Fever Ward in Kenema Sierra Leone
Role: PI
This grant will provide support for a conference to commemorate the opening of a new Lassa Ward at Kenema Government Hospital. The new Ward will replace an historic, but aging, facility and open new opportunities for clinical research.

1 U19 AI109762-01 (PI: Saphire) 3-1-14 – 2-28-19
Consortium for Immunotherapeutics Against Viral Hemorrhagic Fevers
This grant will develop monoclonal antibody (mAb)-based immunotherapeutic products to treat Category A filovirus and arenavirus infections. To do this, we assembled a large, open multidisciplinary consortium from academic and industrial investigators.
Role: Director of Project 3

HHSN272201400003C (PI: Wilson) 1-1-14 – 12-31-16
NIAID
Advanced Development of Flufirvitide-3 for treatment of influenza A and B infections.
This contract will support Phase I human clinical trials of an influenza virus peptide inhibitor.
Role: Director of subcontract.

Competed Research Support (Last 3 years - representative)
R44 AI082778-01 (PI: Wilson) 04/01/09 - 8/31/12
NIAID
Peptide inhibitors of influenza entry-FAST TRACK
Synthetic peptide inhibitors of influenza virus entry will be developed and tested in vitro and in vivo.
Role: Director of subcontract

OVERLAP
Dr. Garry will not accept total funding EFFORT calendar months.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Luis M. Branco	Co-Founder, Zalgen Labs
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Massachusetts, Amherst, MA	B.S.	05/95	Microbiology
Tulane University, New Orleans, LA	Ph.D.	11/10	BioMedical Sciences

A. Personal Statement

The main objective of this proposal is to support a program to establish KGH as a center of excellence for Lassa fever research and training via an International Collaboration in Lassa fever Research (Lassa ICIDR). I am currently serving as an investigator in U01 “Preclinical development of recombinant antigen Lassa fever diagnostics”, BAA “Roles of protective or pathogenic B cell epitopes in human Lassa fever”, R01 “Antibody immunotherapeutics for biodefense against Lassa virus”, and as co-founder of newly established Zalgen Labs, a biotechnology company dedicated to the development of rapid multiplex diagnostics, new vaccine and therapeutic platforms for emerging and neglected viral diseases. I will serve in the Advisory Board of the Lassa ICIDR for this program. I have extensive expertise in multiple facets of research and development of chimeric, humanized, human, and affinity maturation of recombinant antibodies for human use. I have also developed extensive experience in expression of recombinant antigens in a multitude of systems, namely bacterial, baculoviral, and mammalian backgrounds over 24 years, including conceptual design, development, and patenting of a novel, regulatory compliant, and superior mammalian cell-based expression platform for generation of commercial grade proteins (U.S. Patent US8076102). During my 17 years in industry I have been directly involved in the development of licensed antibody therapeutics, such as MedImmune’s Synagis (Palivizumab), Human Genome Sciences’ Benlysta (Belimumab) and ABthrax (raxibacumab), as well as additional antibodies under clinical evaluation (MEDI’s Numax [motavizumab], HGS’ CCR5mAb004, HGS-ETR1 [Mapatumumab]. My research interests in industry also focused on development of industry leading technologies for acceleration of stable cell line development with high regulatory compliance, aimed at reducing timelines and costs in the development path toward IND filings. I continued this work as co-founder of BioFactura, a biotech company in the Washington-D.C. area dedicated to development of immunotherapeutics for Biodefense. The current program will build upon my core expertise and interests in Neglected Tropical Diseases, with a focus on Lassa fever, and a research team including Zalgen co-founder Dr. Robert F. Garry, and a number of distinguished Tulane University, The Scripps Research Institute, Harvard University/BROAD, University of Montana, the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), and Kenema Government Hospital investigators.

B. Positions

Positions and Employment

1989-1995 Laboratory Supervisor and Research Technician, University of Massachusetts, Amherst, MA
1995-2000 Associate Scientist, MedImmune, Gaithersburg, MD
2000-2004 Senior Research Associate/ Manager, Human Genome Sciences, Rockville, MD
2004-2005 Vice President of Research, BioFactura, Rockville, MD
2005-2008 Chief Scientific Officer, BioFactura, Rockville, MD
2008-2013 Program Director, Lassa fever Program, Autoimmune Technologies, LLC, New Orleans, LA
2012-present Co-Founder, Zalgen Labs, New Orleans, LA & Germantown, MD

C. Selected Peer-reviewed Publications

1. Jeffrey G Shaffer, Donald S Grant, John S Schieffelin, Matt L Boisen, Augustine Goba, Jessica N Hartnett, Danielle C Levy, Rachael E Yenni, Lina M Moses, Mohammed Fullah, Mambu Mommoh, Mbalu Fonnie, Richard Fonnie, Lansana Kanneh, Veronica J Koroma, Kandeh Kargbo, Darin Ottomassathien, Ivana J Muncy, Abigail B Jones, Megan M Illick, Peter C Kulakosky, Allyson M Haislip, Christopher M Bishop, Deborah H Elliot, Bethany L Brown, Hu Zhu, Kathryn M Hastie, Kristian G Andersen, Stephen K Gire, Shervin Tabrizi, Ridhi Tariyal, Mathew Stremlau, Alex Matschiner, Darryl B Sampey, Jennifer S Spence, Robert W Cross, Joan B Geisbert, Onikepe A Folarin, Christian T Happi, Kelly R Pitts, F Jon Geske, Thomas W Geisbert, Erica Ollmann Saphire, James E Robinson, Russell B Wilson, Pardis C Sabeti, Lee A Henderson, Sheik Humarr Khan, Daniel G Bausch, **Luis M Branco**, Robert F. Garry. Lassa Fever in Post-conflict Sierra Leone. PLOS Neglected Tropical Diseases, 2014, *in press*.
2. Safronetz D, Sogoba N, Lopez JE, Maiga O, Dahlstrom E, Zivcec M, Feldmann F, Haddock E, Fischer RJ, Anderson JM, Munster VJ, **Branco L**, Garry R, Porcella SF, Schwan TG, Feldmann H. Geographic distribution and genetic characterization of Lassa virus in sub-Saharan Mali. PLoS Negl Trop Dis. 2013 Dec 5;7(12). PMID: 24340119
3. **Luis M Branco**, Jessica N Grove, Matt L Boisen, Jeffrey G Shaffer, Augustine Goba, Mohammed Fullah, Mambu Momoh, Donald S Grant, Robert F Garry. Emerging trends in Lassa fever: redefining the role of immunoglobulin M and inflammation in diagnosing acute infection. *Virology Journal* 2011, 8:478 (24 October 2011). PMID: 22023795
4. **Luis M Branco**, Matt L Boisen, Kristian G Andersen, Jessica N Grove, Lina M Moses, Ivana J Muncy, Lee A Henderson, John S Schieffellin, James E Robinson, James J Bangura, Donald S Grant, Vanessa N Raabe, Mbalu Fonnie, Pardis C Sabeti, Robert F Garry. Lassa Hemorrhagic Fever in a Late Term Pregnancy from Northern Sierra Leone with a Positive Maternal Outcome: Case Report. *Virology Journal* 2011, 8:404 (15 August 2011). PMID: 21843352
5. Jessica N Grove, **Luis M Branco**, Matt L Boisen, Ivana J Muncy, Lee A Henderson, John S Schieffellin, James E Robinson, James J Bangura, Mbalu Fonnie, Randal J Schoepp, Lisa E Hensley, Alhassan Seisay, Joseph N Fair, Robert F Garry, Capacity building permitting comprehensive monitoring of a severe case of Lassa hemorrhagic fever in Sierra Leone with a positive outcome: Case Report. Virology Journal 2011, 8:314 (20 June 2011). PMID: 21689444
6. **Luis M Branco**, Jessica N Grove, Lina M Moses, Augustine Goba, Mohammed Fullah, Mambu Momoh, Randal J Schoepp, Daniel G Bausch, Robert F Garry. Shedding of soluble glycoprotein 1 detected during acute Lassa virus infection in human subjects**.** *Virology Journal* 2010, 7:306 (9 November 2010). PMID: 21062490
7. **Luis M Branco**, Jessica N Grove, Frederick J Geske, Matt L Boisen, Ivana J Muncy, Susan A Magliato, Lee A Henderson, Randal J Schoepp, Kathleen A Cashman, Lisa E Hensley, Robert F Garry. Lassa virus-like particles displaying all major immunological determinants as a vaccine candidate for Lassa hemorrhagic fever**.** *Virology Journal* 2010, 7:279 (20 October 2010). PMID: 20961433
8. Safronetz D, Lopez JE, Sogoba N, Traore' SF, Raffel SJ, Fischer ER, Ebihara H, **Branco L**, Garry RF, Schwan TG, Feldmann H. Detection of Lassa virus, Mali. Emerg Infect Dis. 2010 Jul;16(7):1123-6. PMID: 20587185
9. **Luis M. Branco**, Robert F. Garry. Characterization of the Lassa virus GP1 ectodomain shedding: implications for improved diagnostic platforms. Virology Journal 2009, 6:147 (24 September 2009). PMID: 19778448
10. Megan M. Illick, **Luis M. Branco**, Joseph N. Fair, Kerry A. Illick, Alex Matschiner, Randal Schoepp, Robert F. Garry, Mary C. Guttieri. Uncoupling GP1 and GP2 expression in the Lassa virus glycoprotein complex: implications for GP1 ectodomain shedding. Virology Journal 2008, 5:161 (23 December 2008). PMID: 19105844
11. **Luis M Branco**, Alex Matschiner, Joseph N Fair, Augustine Goba, Darryl B Sampey, Philip J Ferro, Kathleen A Cashman, Randal J Schoepp, Robert B Tesh, Daniel G Bausch, Robert F Garry, Mary C Guttieri. Bacterial-based systems for expression and purification of recombinant Lassa virus proteins of immunological relevance. Virology Journal 2008, 5:74 (6 June 2008). Virol J. 2008. PMID: 18538016
12. John P. DeVincenzo, Caroline B. Hall, David W. Kimberlin, Pablo J. Sanchez, William J. Rodriguez, Barbara A. Jantaush, Lawrence Corey, Jeffrey S. Khan, Janet A. Englund, JoAnn A. Suzich, Fran J. Palmer-Hill, **Luis Branco**, Syd Johnson, Nita K. Patel, Franklin M. Piazza. Surveillance of Clinical

Respiratory Syncytial Virus (RSV) Isolates for Palivizumab (Synagis®) Resistant Mutants. J. Infect. Dis. 2004; 190:975-978. PMID: 15295704

13. **Branco L**, Barren P, Mao SY, Pfarr D, Kaplan R, Postema C, Langermann S, Koenig S, and Johnson S. Selective Deletion of Antigen-Specific, Activated T Cells by a Humanized mAb to CD2 (MEDI-507) is Mediated by NK Cells. Transplantation 1999; 68 (10): 1588-1596. PMID: 10589960
14. Shaffer JG, Grant D Sand Schieffelin JS *et al.* Lassa fever in post-conflict Sierra Leone. PLoS Neglected Tropical Diseases 2014, in press.

D. Research Support

Ongoing Research Support

BAA NIAID-DAIT-NIHAI2008031 Robinson (PI) 09/30/2009 – 09/29/2014
NIH/NIAID BAA
B cell epitope discovery and mechanisms of antibody protection
Role: investigator

1 U01 AI082119-01 Garry (PI) 04/30/2009 – 04/29/2014
NIH/NIAID
Recombinant antigen multiagent diagnostic assays for Lassa and other arenaviruses
Role: investigator

1 R01 AI104621-01 Garry (PI) 02/27/2013 – 01/31/2018
NIH/NIAID
Antibody immunotherapeutics for biodefense against Lassa virus
Role: investigator

Completed Research Support

1R43AI088843-01 Lopez (PI) 04/01/2010 – 03/31/2012
NIH/NIAID SBIR
Recombinant antigen diagnostics for filoviruses
Role: investigator

1 UC1 AI067188-01 Garry (PI) 09/30/2005 - 09/29/2008
NIH/ NIAID
Recombinant antigen multiagent diagnostic assays for Lassa and other arenaviruses
Role: Co-Investigator

Department Of Defense SBIR Phase III Contract No.:
W81XWH-06-C-0029 Branco (PI) 07/21/2008 – 08/29/2010
Development of a Therapeutic Human Antibody Cocktail Countermeasure Against Biowarfare Poxviridae Threat Agents

Department Of Defense SBIR Phase II Contract No.:
W81XWH-06-C-0029 Branco (PI) 10/14/2006 – 10/13/2008
Generation of Stable Eukaryotic Cell Lines Expressing High Yields of Therapeutic Human Antibodies Against Biowarfare Viral Threat Agents

Private Source

Private Source (Jan 17, 2007) Branco (PI) Q1 2007 – Q1 2008
StableFast™ Mammalian Expression and Manufacturing System Development

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

Kathleen A. Cashman	POSITION TITLE Investigator, Geneva Foundation, Virology Division, USAMRIID
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Wright State University, Dayton, OH	BS	1995	Environmental Science
California State University, Long Beach, CA	MS	2000	Microbiology
Georgetown University, Washington, DC	PhD	2005	Microbiology & Immunology
National Research Council Research Associateship at USAMRIID	Postdoctoral Training	2005-2009	

A. Personal Statement

Since joining USAMRIID in 2005, I have been engaged in the development and testing of DNA-based vaccines against arenavirus infections and the development and characterization of the animal models supporting the vaccine research endeavors. At USAMRIID, I am currently engineering, refining and testing a DNA vaccine that has been shown to be 100% protective in both guinea pig and primate models of Lassa fever. This vaccine is moving forward for further development with the end goal of preparing the vaccine for use in humans. I have over eight years of experience working in a biosafety level 4 (BSL-4) laboratory. I have direct experience designing, coordinating and conducting both *in vitro* and *in vivo* vaccine and therapeutic efficacy studies in the guinea pig and nonhuman primate models of arenavirus hemorrhagic fever. I have been involved in fruitful collaborations with industry and academic partners for basic research, therapeutics development and efficacy studies, and vaccine development.

B. Positions and Honors

Professional Positions:

2013 – Present	Principal Investigator, Geneva Foundation, Virology Division, USAMRIID
2009 – 2013	Investigator, Team Ke'aki Tech, Virology Division, USAMRIID
2008 – 2009	Senior Research Associate, National Academy of Sciences, National Research Council
2005 – 2008	Research Associate, National Academy of Sciences, National Research Council

Awards:

2011	Outstanding Research Award – DNA Vaccines 2011 Conference, San Diego, CA.

C. Selected peer-reviewed publications (in chronological order)

Ifon ET, Pang AL, Johnson W, Cashman KA, Zimmerman SK, Muralidhar S, Chan WY, Casey J, Rosenthal LJ. 2005. U94 alters FN1 and ANGPTL4 gene expression and inhibits tumorigenesis of prostate cancer cell line PC3. Cancer Cell Int. 22(5):19-24.

Gandy SZ, Linnstaedt SD, Muralidhar S, Cashman KA, Rosenthal LJ, Casey JL. RNA Editing of the HHV-8 Kaposin Transcript Eliminates Its Transforming Activity and Is Induced During Lytic Replication. J Virol Oct 3, 2007 (epub ahead of print).

Branco L, Matschiner A, Fair JN, Goba A, Ferro P, Cashman KA, Sampey D, Schoepp R, Tesh R, Garry F, Guttieri MC. 2008. Mammalian-and Bacterial-Based Systems for Expression and Purification of Recombinant Lassa Virus Proteins of Immunological Relevance. Virol J Jun 6; 5:74.

Cashman KA, Smith MA, Twenhafel NA, Larson RA, Jones KF, Allen RD 3rd, Dai D, Chinsangaram J, Bolken TC, Hruby DE, Amberg SM, Hensley LE, Guttieri MC. 2011. Evaluation of Lassa antiviral compound ST-193 in a guinea pig model. Antiviral Res. 2011 Apr; 90(1):70-9.

Twenhafel NA, Mattix ME, Johnson JC, Robinson CG, Pratt WD, Cashman KA, Wahl-Jensen V, Terry C, Olinger GG, Hensley LE, Honko AN. Pathology of experimental aerosol Zaire ebolavirus infection in rhesus macaques. Vet Pathol. 2013 May; 50(3):514-29.

Cashman KA, Broderick KE, Wilkinson ER, Shaia CI, Bell TM, Shurtleff AC, Spik KW, Badger CV, Guttieri MC, Sardesai NY, Schmaljohn CS. Enhanced Efficacy of a Codon-Optimized DNA Vaccine Encoding the Glycoprotein Precursor Gene of Lassa Virus in a Guinea Pig Disease Model When Delivered by Dermal Electroporation. *Vaccines*. 2013; 1(3):262-277.

Bell TM, Shaia CI, Bunton TE, Robinson CG, Wilkinson ER, Hensley LE, and Cashman KA. 2014. Comparison of Pathology of Experimental Machupo virus Infection, Chicava strain, in Cynomolgus Macaques by Intramuscular and Aerosol Exposure Routes. Submitted to Veterinary Pathology, in review.

D. Research Support

Ongoing Research Support:

1R01AI105383-01 (Schmaljohn) 04/15/2013 - 03/31/2018
NIH
Preclinical Assessment of a Multi-Head Electroporation Device for Delivery of Biodefense DNA Vaccines
This proposal seeks to further the preclinical development of a DNA-based vaccine delivery platform including integrated and automated intradermal electroporation delivery. First-of-their-kind devices will be engineered and tested with a vaccine against Lassa virus, a Category A pathogen. The improved vaccine platform will be suitable for mass vaccination using multiagent vaccines.
Role: Co-Investigator

Completed Research Support:

N/A

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Donald S. Grant	Medical Officer, Kenema Government Hospital Lassa Fever Program
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
College of Medicine and Allied Health Sciences, University of Sierra Leone	M.B.Ch.B	07/08	Medicine

A. Personal Statement

Dr. Donald Grant is the Deputy Chief Physician of the Lassa ward at the Kenema Government Hospital, a position he took over in October 2010. Trained in Freetown, Sierra Leone, Dr. Grant is also a lecturer in the Community Health Department of the College of Medicine and Allied Health Sciences at the University of Sierra Leone. Along with his clinical work in the Lassa ward, Dr. Grant has been an investigator for a wide range of NIH-sponsored research studies, including a current study that conducts a population genetics based study of genetic resistance and susceptibility to Lassa fever. He is also the Principal Investigator of the PEER Health Grant on Lassa Fever pathobiology in Children and During Pregnancy. With the upcoming breaking ground of the new Lassa ward Dr. Grant looks forward to his future involvement with research and understanding the transmission and infection of the virus that causes Lassa Fever, and the basis for susceptibity and resistance to this and other pathogens.

B. Positions and Honors.

2009-2011 Assistant Secretary General, Sierra Leone Medical and Dental Association (SLMDA)
2009-date Lecturer, Department of Community Health, College of Medicine and Allied Health Sciences, University of Sierra Leone
2008-date Trainer of a Health Internetwork Access to Research Initiative (HINARI). Training for final year medical students of the College of Medicine and Allied Health Sciences, University of Sierra Leone
2009-date Member of American Society of Microbiology
2010-date Physician In-Charge of the Lassa Fever Isolation Unit, Kenema Government Hospital, Ministry of Health and Sanitation
2011-date Member of the Viral Hemorrhagic Fever Consortium
2011-date The Fundamentals of International Clinical Research Workshop, Division of Microbiology & Infectious Diseases, NIAID, NIH
2012-date Member of the America Society for Tropical Medicine and Hygiene

C. Selected peer-reviewed publications (in chronological order).

Branco, L.M., Grove, J.N., Boisen, M.L., Shaffer, J.G., Fullah, M., Goba, A., Momoh, M., Grant, D.S., Garry, R.F. (2011). Emerging trends in Lassa fever: redefining the role of immunoglobulin M and inflammation in diagnosing acute infection. *Virology Journal*. 8, 478.

Branco, L.M., Boisen, M.L., Andersen, K.G., Grove, J.N., Moses, L.M., Muncy, I.J., Henderson, L.A., Schieffellin, J.S., Robinson, J.E., Bangura, J.J., Grant, D.S., Raabe, V.N., Fonnie, M., Sabeti, P.C., Garry, R.F. (2011). Lassa hemorrhagic fever in a late term pregnancy from northern Sierra Leone with a positive maternal outcome: case report. *Virology Journal*. 8, 480.

Shaffer JG, Grant D Sand Schieffelin JS *et al.* Lassa fever in post-conflict Sierra Leone. PLoS Neglected Tropical Diseases 2014, in press.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Sheik Humarr Khan	Physician Specialist, Internal Medicine, Ministry Of Health and Sanitation, Freetown , Sierra Leone
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown	M.B.Ch.B	07/01	Medicine
West African College of Physicians, Accra, Ghana	MWACP	07/13	Internal Medicine

A. Personal Statement

Dr. Sheik Humarr Khan, is a product of the College Of Medicine and Allied Health Sciences (COMAHS), University of Sierra Leone (USL). He graduated with a Bachelors of medicine and Bachelors in surgery (MBChB) in 2001. Being a budding tropical medicine/infectious disease Physician at the time, Dr. Khan was recruited as a medical officer at the Directorate of Disease Prevention and Control, Ministry Of Health and Sanitation (MOHS). He served in this place for almost two years until 2005 when, perhaps with his strong sense of character, he was appointed by the MOHS as the Chief physician Lassa fever Program, Kenema Government Hospital (KGH), Sierra Leone. Dr. Khan stepped into the shoes of his predecessor, the late Dr. Aniru Conteh who unfortunately died of the Lassa fever. In his capacity as the Chief Physician of the Lassa Fever Program, KGH, Dr. Khan was concurrently contracted by then United Nations Mission in Sierra Leone (UNAMSIL) as a contract physician and consultant for Lassa fever in Sierra Leone. This appointment was a morale booster to the Pakistani Armed contingent battalion and Zambian contingent in this part of the country, who were almost packing their bags to leave in the wake of the death of Dr. Khan's predecessor for fear that there will not be any other Physician to take care of them having already suffered some few fatalities from this disease. From 2005-2010 he was Physician In-charge, HIV/AIDS services, Kenema Government Hospital (KGH), Kenema Eastern Sierra Leone, and from 2006-2010Physician Consultant, Mano River Union Lassa fever network, WHO/Tulane University. In his quest to realize his full clinical, academic and research potentials he gained entrance at the Korle-Bu Teaching Hospital (KBTH), Accra, Ghana where he went through his residency training in the field internal medicine for a period of almost three years and was awarded the degree of MWACP (2013). He is back to his field of work as the In-Charge of the Lassa Fever Program, KGH, MOH. In January, 2014, he was appointed as Associate Lecturer, Department of Medicine, COMAHS, USL. Today, Dr Sheik Humarr Khan, is one of the world's leading experts in the clinical care of viral hemorrhagic fevers. He is well prepared to lead the Lassa ICIDR.

B. Positions and Honors.

2001 – Present Member of the Sierra Leone Medical and Dental Association
2002 - Present Certificate of Knowledge in HIV/AIDS and Human Resource Building Capacity, Africa AIDS Research Network
2006 – 2010. Part-time Lecturer, Diploma in Public Health, East Polytechnic Institution, Njala University College, University of Sierra Leone

2006 Best Medical Physician in Kenema District, Awarded by the Kenema Youth Association

2007 – Present Member, U.S. National Institutes of Health Clinical Working Group on Viral Hemorrhagic Fevers

2008 Participant: Regional HIV/AIDS training care and treatment workshop, Komfo Anokye Teaching Hospital, Kumasi, Ghana, February 18-29, 2008

2009 – Present Member, World Health Organization Clinical Working Group on Viral Hemorrhagic Fevers

2013- Present. Cert. , Update Course in Neurology, WACP/RCP, Lagos, Nigeria.

C. Selected peer-reviewed publications (in chronological order).

Khan SH, Goba A, Chu M, Roth C, Healing T, Marx A, Fair J, Guttieri MC, Ferro P, Imes T, Monagin C, Garry RF, DG Bausch for the Mano River Union Lassa Fever Network (2008). New opportunities for field research on the pathogenesis and treatment of Lassa fever. *Antiviral Res* 78 (1):103-115

Kouyoumdjian FG, Seisay AL, Kargbo B, **Khan SH** (2010). The Voluntary HIV Counselling and testing Service in Kenema District, Sierra Leone, 2004-2006: a descriptive study. *BMC Int Health Hum Rights*. 9;10:4

Bausch DG, Moses LM, Goba A, Grant D, and **H Khan**. Lassa Fever. In SK Singh (ed): Viral Hemorrhagic Fevers, Taylor and Francis Group/ CRC Press, Boca Raton, FL (in Press)

Hadi CM, Goba A, **Khan SH**, Bangura J, Sankoh Mbalu, Koroma S, Juana B, Bah A, Coulibaly M, and Bausch DG: Ribavirin for Lassa Fever Postexposure Prophylaxis - Emerging Infectious Diseases. www. cdc.gov/eid. Vol .16, No. 12, December 2010

Shaffer JG, Grant D Sand Schieffelin JS …Garry RF. Lassa fever in post-conflict Sierra Leone. PLoS Neglected Tropical Diseases 2014, in press.

BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Sheik Humarr Khan	Physician Specialist, Internal Medicine, Ministry Of Health and Sanitation, Freetown , Sierra Leone
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown	M.B.Ch.B	07/01	Medicine
West African College of Physicians, Accra, Ghana	MWACP	07/13	Internal Medicine

A. Personal Statement

Dr. Sheik Humarr Khan, is a product of the College Of Medicine and Allied Health Sciences (COMAHS), University of Sierra Leone (USL). He graduated with a Bachelors of medicine and Bachelors in surgery (MBChB) in 2001. Being a budding tropical medicine/infectious disease Physician at the time, Dr. Khan was recruited as a medical officer at the Directorate of Disease Prevention and Control, Ministry Of Health and Sanitation (MOHS). He served in this place for almost two years until 2005 when, perhaps with his strong sense of character, he was appointed by the MOHS as the Chief physician Lassa fever Program, Kenema Government Hospital (KGH), Sierra Leone. Dr. Khan stepped into the shoes of his predecessor, the late Dr. Aniru Conteh who unfortunately died of the Lassa fever. In his capacity as the Chief Physician of the Lassa Fever Program, KGH, Dr. Khan was concurrently contracted by then United Nations Mission in Sierra Leone (UNAMSIL) as a contract physician and consultant for Lassa fever in Sierra Leone. This appointment was a morale booster to the Pakistani Armed contingent battalion and Zambian contingent in this part of the country, who were almost packing their bags to leave in the wake of the death of Dr. Khan's predecessor for fear that there will not be any other Physician to take care of them having already suffered some few fatalities from this disease. From 2005-2010 he was Physician In-charge, HIV/AIDS services, Kenema Government Hospital (KGH), Kenema Eastern Sierra Leone, and from 2006-2010Physician Consultant, Mano River Union Lassa fever network, WHO/Tulane University. In his quest to realize his full clinical, academic and research potentials he gained entrance at the Korle-Bu Teaching Hospital (KBTH), Accra, Ghana where he went through his residency training in the field internal medicine for a period of almost three years and was awarded the degree of MWACP (2013). He is back to his field of work as the In-Charge of the Lassa Fever Program, KGH, MOH. In January, 2014, he was appointed as Associate Lecturer, Department of Medicine, COMAHS, USL. Today, Dr Sheik Humarr Khan, is one of the world's leading experts in the clinical care of viral hemorrhagic fevers. He is well prepared to lead the Lassa ICIDR.

B. Positions and Honors.

2001 – Present Member of the Sierra Leone Medical and Dental Association
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2009 – Present Member, World Health Organization Clinical Working Group on Viral Hemorrhagic Fevers

2013- Present. Cert. , Update Course in Neurology, WACP/RCP, Lagos, Nigeria.

C. Selected peer-reviewed publications (in chronological order).

Khan SH, Goba A, Chu M, Roth C, Healing T, Marx A, Fair J, Guttieri MC, Ferro P, Imes T, Monagin C, Garry RF, DG Bausch for the Mano River Union Lassa Fever Network (2008). New opportunities for field research on the pathogenesis and treatment of Lassa fever. *Antiviral Res* 78 (1):103-115

Kouyoumdjian FG, Seisay AL, Kargbo B, **Khan SH** (2010). The Voluntary HIV Counselling and testing Service in Kenema District, Sierra Leone, 2004-2006: a descriptive study. *BMC Int Health Hum Rights*. 9;10:4

Bausch DG, Moses LM, Goba A, Grant D, and **H Khan**. Lassa Fever. In SK Singh (ed): Viral Hemorrhagic Fevers, Taylor and Francis Group/ CRC Press, Boca Raton, FL (in Press)

Hadi CM, Goba A, **Khan SH**, Bangura J, Sankoh Mbalu, Koroma S, Juana B, Bah A, Coulibaly M, and Bausch DG: Ribavirin for Lassa Fever Postexposure Prophylaxis - Emerging Infectious Diseases. www. cdc.gov/eid. Vol .16, No. 12, December 2010

Shaffer JG, Grant D Sand Schieffelin JS …Garry RF. Lassa fever in post-conflict Sierra Leone. PLoS Neglected Tropical Diseases 2014, in press.

BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Lina Michiko Moses	Research Scientist, Department of Microbiology and Immunology, Tulane University
eRA COMMONS USER NAME eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Utah State University, Logan, Utah	BS	1994-2000	Anthropology
Tulane University School of Public Health and Tropical Medicine, New Orleans, Louisiana	MSPH	2004-2005	Tropical Medicine
Tulane University School of Public Health and Tropical Medicine, New Orleans, Louisiana	PhD	2006-2012	Tropical Medicine, Community Health, Environmental Health

A. Personal Statement

My primary interest is in the disease ecology of viral zoonoses. I utilize population biology, ecological and epidemiological methods to understand macro-level pathogen/host interactions and monitor reservoir host populations. The ultimate goal of my research is to develop community-level interventions to halt animal-to-human transmission.

B. Positions and Honors

1997 – 1998. Research Coordinator. Department of Genetic Epidemiology, University of Utah, Salt Lake City, Utah, USA. PI: Lisa Cannon-Albright, PhD.

1999 – 2002. Research Coordinator. Department of Neurology, Oregon Health & Sciences University, Portland, Oregon, USA. PI: Haydeh Payami, PhD.

2002 – 2004. Research Coordinator. Division of Genetic Disorders, Wadsworth Center, New York State Department of Health, Albany, New York, USA. PI: Haydeh Payami, PhD

2004 – 2006. Research Coordinator, volunteer. Department of Tropical Medicine, Tulane University, New Orleans, Louisiana, USA. PI: Daniel G. Bausch, MD MPH&TM.

2011 – present. Research Scientist—Ecology and Community-based Research. Tulane Lassa Fever Program, Department of Microbiology & Immunology, Tulane University, New Orleans, Louisiana, USA. PI: Robert F. Garry, PhD.

2006-2010 Louisiana Board of Regents Doctoral Fellowship in Public Health for Transdisciplinary Research

2010 Dean of the School of Public Health and Tropical Medicine Award for Excellence in Research and Presentation by a Public Health Doctoral Student, Tulane University Health Sciences Research Days.

2013 Tulane University School of Public Health & Tropical Medicine Alumni Association Student Recognition Award.

Other Professional Activities

Practicum supervisor for one undergraduate and three MSPH students, Sierra Leone, 2009.

Teaching Assistant. Tulane School of Public Health & Tropical Medicine, New Orleans, Louisiana, USA. 2006 – 2009. Courses: CHSC 651-Contemporary Issues in Maternal and Child Health, CHSC 612-Monitoring and Evaluation of Health Education & Communication, TRMD 642-Tropical Virology, TRMD 680-Emerging Pathogens.

Member, American Society of Tropical Medicine and Hygiene, 2005 – present.

Member, International Society for Infectious Diseases, 2010 – present.

Member, American Society for Microbiology, 2013 – present

Ad Hoc Reviewer, *Vector-Borne and Zoonotic Diseases Journal*

C. Selected Publications

Peer-Reviewed Publications

1. Poorkaj P, **Moses L,** Montimurro JS, Nutt JG, Schellenberg GD, Payami H. *parkin* mutation dosage and the phenomenon of anticipation: a molecular genetic study of familial parkinsonism. *BMC Neurology* 2005, 5(1): 4-7.
2. Payami H, Zhu M, Montimurro J, Keefe R, McCulloch C, **Moses L**. One step closer to fixing association studies: evidence for age and gender specific allele frequency variation and deviations from Hardy Weinberg expectations in controls. *Human Genetics* 2005 Dec, 118(3-4):322-30.
3. Kay DM, Moran D, **Moses L**, Poorkaj P, Zabetian CP, Nutt J, Factor SA, Yu CE, Montimurro JS, Keefe RG, Schellenberg GD, Payami H. Heterozygous *parkin* point mutations are as common in control subjects as in Parkinson's patients. *Annals of Neurology* 2007 Jan,61(1):47-54.
4. Khan SH, Goba A, Chu M...**Moses LM**…Mano River Union Lassa Fever Network. New opportunities for field research on the pathogenesis and treatment of Lassa fever. *Antiviral Research* 2008 Apr, 78(1):103-15.
5. Kay DM, Stevens CF, Hamza TH, Montimurro JS, Zabetian CP, Factor SA, Samii A, Griffith A, Roberts JW, Holho ES, Higgins DS, Gancher S, **Moses L**, Zareparsi S, Poorkaj P, Bird T, Nutt J, Schellenberg GD, Payami H. A comprehensive analysis of deletions, multiplications, and copy number variations in PARK2. *Neurology* 2010 Sep 28, 75(13):1189-94.
6. Branco LM, Grove JN, **Moses LM**, Goba A, Fullah M, Momoh M, Schoepp RJ, Bausch DG, Garry RF. Shedding of soluble glycoprotein 1 detected during acute Lassa virus infection in human subjects. *Virology Journal* 2010, 7(1): 306.
7. Branco LM, Boisen ML, Andersend KG, Grove JN, **Moses LM**, Muncy IJ, Henderson LA, Schieffelin JS, Robinson JE, Bangura JJ, Grant DS, Raabe VN, Fonnie M, Zaitsev EM, Sabeti PC, Garry RF. Lassa hemorrhagic fever in a late term pregnancy from northern Sierra Leone with a positive maternal outcome: case report. *Virology Journal* 2011,8(1):404.
8. MacNeil A, Abel J, Reynolds MG, Lash RR, Fonnie R, Kanneh LD, Robert W, Lungay VK, Goba A, **Moses LM**, Damon IK, Karem K, Bausch DG. Serologic evidence of human orthopoxvirus infections in Sierra Leone. *BMC Research Notes* 2011, 4(1): 465.
9. Bond NG, Schieffelin J, **Moses L,** Bennett A, Bausch DG. Historical look at the first reported cases of Lassa fever: IgG antibodies 40 years after acute infection. *Am J Tropical Medicine & Hygiene 2013*, 88(2):241-244.
10. Kelly JD, Barrie MB, Ross RA, Temple B, **Moses LM**, Bausch DG. Housing equity for health equality—a rights-based approach to the control of Lassa fever in post-war Sierra Leone. *BMC International Health and Human Rights*, 2013, 13:2 (2 January 2013).
11. Shaffer JG, Grant D Sand Schieffelin JS *et al.* Lassa fever in post-conflict Sierra Leone. PLoS Neglected Tropical Diseases 2014, in press.

Book Chapters

1. Bond NG, **Moses LM**, Peterson AT, Mills JN, Bausch DG. “Environmental Aspects of the Viral Hemorrhagic Fevers.” In: *The Praeger Handbook of Environmental Health*. ABC-CLIO: Santa Barbara, CA, April 2012).
2. Bausch DG, **Moses LM**, Goba A, Grant DS, Khan H. “Lassa Fever.” In: *Viral Hemorrhagic Fevers* (Singh SK, Ruzek D, eds). Taylor & Francis (CRC PR I LLC), *in press*.

D. Research Support:
Ongoing Research Support

NE/J001570/1 M. Leach (PI) 2/1/12-8/1/15
Dynamic Drivers of Disease in Africa Consortium
Private Source

Dynamic Drivers of Disease in Africa is a research program designed to deliver cross-cutting science on the relationships between ecosystems, zoonoses, health and wellbeing with the objective of helping people move out of poverty and promoting social justice. The 3.5-year, £3.2m program focuses on four emerging or re-emerging zoonotic diseases in four diverse African ecosystems. Subcontract Scope of Work: The overall case study will examine climatological, ecological, ecosystem services, land-use and demographic parameters of Lassa virus transmission in four villages in eastern Sierra Leone. Dr. Moses coordinates research activities between in-country investigators and directs ecology-based investigations.
Role: Country Field Coordinator % effort

Private Source R. Garry (PI) 8/15/12-2/28/14
Evidence-based Lassa Fever Prevention and Integration into Kenema District WASH Programmes
Source: Private Source
Implementation of an intervention trial of three rodent control methods designed to reduce risk of Lassa fever incidence in rural Sierra Leone. Integrate of Lassa fever prevention and control into regional and national level sanitation and hygiene plans and policy. Dr. Moses directs the overall project.
Role: Co-Investigator % effort

1U01AI082119-03 Garry (PI) 4/01/09-3/31/14
Source: NIAID
Pre-Clinical development of recombinant antigen diagnostics for Lassa Fever
Source: National Institutes of Health
The goal of this project is to develop, validate and market rapid diagnostic tests for Lassa Hemorrhagic Fever. Dr. Moses supervises community sample and data collection.
Role: Investigator % effort

HHSN272200900049C J. Robinson (PI) 0/30/09-9/29/14
Source: NIAID-DAIT
Roles of Protective or Pathogenic B Cell Epitopes in Human Lassa Fever
The goals are to discover novel B cell epitopes of Lassa virus protein antigens and to elucidate mechanisms of antibody-mediated protection or pathogenesis in a well-characterized cohort of persons exposed to diverse strains of Lassa virus at different stages and with different severities of Lassa fever. Dr. Moses supervises community sample and data collection.
Role: Investigator % effort

Completed Research Support
LEQSF (2005-10)-GF-18 R. Oberhelman (PI) 8/1/06-7/31/10
Source: Louisiana State Board of Regents
This grant provides stipend support for four years of graduate-level research training.
Role: Doctoral Student % effort

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Jack H. Nunberg	Director, Montana Biotechnology Center Professor, Biological Sciences
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Cornell University, Arts and Sciences, Ithaca, NY	AB	06/72	Biology and Chemistry
Stanford University, Stanford, CA	PhD	06/79	Biophysics

A. Personal statement. My scientific career includes biotechnology/pharmaceutical R&D and, more recently, academia. I have been at The University of Montana since 1996 as Director of the Montana Biotechnology Center and Professor of Biological Sciences. The Biotechnology Center operates as a stand-alone department reporting to the Vice President for Research, with a mission to facilitate biomedical and biotechnology research across colleges and departments. This administrative experience prepares me for the shared responsibilities of serving on the Lassa ICIDR Advisory Board. My scientific project management experience at Cetus, Merck Research Labs and Genentech, as well as a record of continuous research funding at UM, speaks to my ability to lead complex research and development programs. My laboratory focuses on virus entry and its inhibition, initially with HIV-1 and for the past 9 years with the arenavirus envelope glycoprotein GPC. During this latter period, we have published 19 articles that have characterized structure-function relationships that promote GPC's pH-dependent membrane-fusion activity and its inhibition by small-molecule fusion inhibitors discovered by SIGA Technologies and the Scripps Research Institute. We have also collaborated with Dr. Brian Gowen and Toyama Chemical Co. to investigate the molecular basis for T-705 (favipiravir) inhibition of the arenavirus RNA-dependent RNA polymerase and identified and characterized the intracellular structures organized by arenaviruses for their replication. In addition to my administrative and research experience and proven track record, I am also in a position to contribute to the development of drug development infrastructure based on my work at Cetus (Project Manager, New Animal Drug Application for veterinary testing of PROLEUKIN interleukin-2), Merck (discovery and development leading to 2-pyridinone non-nucleoside-analog HIV-1 RT inhibitors and CRIXIVAN HIV-1 protease inhibitor) and Genentech (HIV AIDSVAX gp120 vaccine).

B. Positions and Honors.

Positions and Employment

1979 to 1985 Scientist, Associate Scientist, Cetus Corporation, Emeryville, CA.
1985 to 1989 Senior Scientist, Cetus Corporation, Emeryville, CA.
1989 to 1991 Senior Research Fellow, Virus and Cell Biology, Merck Research Labs, West Point, PA.
1991 to 1994 Senior Scientist, Immunology, and Head, Research Virology Laboratory. Genentech, South San Francisco, CA.
1994 to 1996 Consultant, Chiron Corporation. Emeryville, CA.
1996 to present Professor of Biological Sciences; Director, Montana Biotechnology Center. The University of Montana, Missoula, MT.

2011 to present Member, NIH Virology A Study Section (VIRA)

C. Selected peer-reviewed publications (in chronological order).

1. York J, Romanowski V, Lu M, and Nunberg JH. (2004) The signal peptide of the Junín arenavirus envelope glycoprotein is myristoylated and forms an essential subunit of the mature G1-G2 complex. J. Virology

78:10783-10792. PMC516395
2. York J and Nunberg JH. (2006) Role of the stable signal peptide of Junín arenavirus envelope glycoprotein in pH-dependent membrane fusion. J. Virology 80:7775-80. PMC1563716
3. Radoshitzky SR, Abraham J, Spiropoulou CF, Kuhn JH, Nguyen D, Li W, Nagel J, Schmidt PJ, Nunberg JH, Andrews NC, Farzan M and Choe H. (2007) Transferrin receptor 1 is an obligate receptor for New World hemorrhagic fever arenaviruses. Nature 446: 92-6. PMC3197705
4. Agnihothram SS, York J, Trahey M and Nunberg JH. (2007) Bitopic membrane topology of the stable signal peptide in the tripartite Junín virus GP-C envelope glycoprotein complex. J Virology 81:4331-7. PMC1866146
5. York J. and Nunberg JH. (2007) A novel zinc-binding domain is essential for formation of the functional Junín virus envelope glycoprotein complex. J Virology 81:13385-91. PMC2168868
6. Lee AM, Rojek JM, Spiropoulou CF, Gundersen AT, Jin W, Shaginian A, York J, Nunberg JH, Boger DL, Oldstone MB and Kunz S. (2008) Unique small molecule entry inhibitors of hemorrhagic fever arenaviruses. J Biol Chem. 283:18734-42. PMC2441566
7. York J, Dai D, Amberg SM and Nunberg JH. (2008) pH-induced activation of arenavirus membrane fusion is antagonized by small-molecule inhibitors. J Virol 82:10932-9. PMC2573205
8. York J and Nunberg JH (2009) Intersubunit interactions modulate pH-induced activation of membrane fusion by the Junín virus envelope glycoprotein GPC. (2009) J. Virol. 83: 4121-6. PMC2668491
9. York J, Berry JD, Ströher U, Li Q, Feldmann H, Lu M, Trahey M, Nunberg JH. (2010) An antibody directed against the fusion peptide of Junin virus envelope glycoprotein GPC inhibits pH-induced membrane fusion. *J Virology 84*:6119-29. PMC2876654
10. Briknarova K, Thomas CJ, York J, Nunberg JH. (2011) Structure of a zinc-binding domain in the Junin virus envelope glycoprotein. J Biol Chem. 286:1528-36. PMC3020761
11. Mendenhall M, Russell A, Juelich T, Messina EL, Smee DF, Freiberg AN, Holbrook MR, Furuta Y, de la Torre JC, Nunberg JH, Gowen BB. (2011) T-705 (favipiravir) inhibition of arenavirus replication in cell culture. Antimicrob Agents Chemother. 55:782-7. PMC3028760
12. Thomas CJ, Casquilho-Gray, HE, York J, DeCamp DL, Dai D, Petrilli EB, Boger DL, Slayden RA, Amberg SM, Sprang SR, Nunberg JH. (2011) A specific interaction of small-molecule entry inhibitors with the envelope glycoprotein complex of the Junín hemorrhagic fever arenavirus. J Biol Chem 286:6192-200. PMC3057843
13. Messina EL, York J, Nunberg JH. (2012) Dissection of the role of the stable signal peptide of the arenavirus envelope glycoprotein in membrane fusion. J Virol. 86:6138-45. PMC3372177
14. Baird NL, York J, Nunberg JH. (2012) Arenavirus infection induces discrete cytosolic structures for RNA replication. J Virol. 86:11301-10. PMC3457164
15. Thomas CJ, Shankar S, Casquilho-Gray, HE, York J, Sprang SR, Nunberg JH. (2012) Biochemical reconstitution of hemorrhagic-fever arenavirus envelope glycoprotein-mediated membrane fusion. PLoS ONE 7:e51114. PMC3511403

D. Research support.

Active Research Support

R01 AI074818; Nunberg (PI) 04/01/08 – 03/31/14 (no cost)
NIH
Structure-Function studies of the Tripartite Junín arenavirus GPC
This project will study the tripartite envelope glycoprotein complex GPC, its pH-dependent membrane-fusion activity, and the role of GPC and the intersubunit zinc finger in virion assembly and morphogenesis.

U54 AI065357; Belisle, Colorado State Univ (PI) 05/01/09 – 04/30/14
NIH/Rocky Mountain Regional Center of Excellence for Biodefense and Emerging Infections - Subaward
Arenavirus entry and its inhibition – Nunberg (PL)
This project will characterize the amino-acid and pharmacophore determinants for inhibition by small-molecule fusion inhibitors discovered by SIGA. Biophysical measures of inhibitor binding and computational methods to define the core pharmacophore will support the identification of a potent and broadly active inhibitor for small-animal model studies.

R21 AI099870; Nunberg (PI) 02/01/12 – 01/31/15 (no cost)
NIH
Molecular determinants of host-cell interactions required for arenavirus replication
This project examines the virus-host interplay in the establishment and function of the cytosolic sites of arenavirus replication and transcription.

Completed Research Support

R01 AI093387; Amberg/SIGA Technologies (PI) 09/01/11 – 08/31/13
NIH/Partnerships for Biodefense
Antiviral drugs for arenaviruses
This Partnership for Biodefense award seeks to develop SIGA Technology lead arenavirus entry inhibitors through preclinical phases. The subaward to UM/Nunberg is focused on model-driven lead optimization and inhibitor-binding assessments during years 1 and 2 of the project.

U54 AI065357; Belisle, Colorado State Univ (PI) 05/01/09 – 04/30/12
NIH/RMRCE Subaward
T-705 pyrazine derivative treatment of highly pathogenic arenaviral infections
Continuing Research Project, Nunberg (co-PL)
This project seeks to advance the development of T-705 (Toyama Chemical, Inc) for use in the treatment of arenaviral hemorrhagic fevers.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Sabeti, Pardis C.	Associate Professor, Harvard University Center for Systems Biology, Department of Organismic and Evolutionary Biology
eRA COMMONS USER NAME eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
M.I.T., Cambridge, MA	B.S.	1997	Biology
University of Oxford, Oxford, UK	M.Sc.	1998	Human Biology
University of Oxford, Oxford, UK	D.Phil.	2003	Biological Anthropology
Harvard Medical School, Boston, MA	M.D.	2006	Medicine
Broad Institute of MIT and Harvard, Cambridge, MA	Postdoc	2006	Computational Genetics

A. Personal Statement

With a background in both computational genetics and medicine, my research interests have been to understand the mechanisms of evolutionary adaptation in humans and microbial pathogens. We seek to understand both host and microbial genetic drivers of susceptibility, as well as mechanisms by which the microbes continue to evolve to evade our immune systems, vaccines, and therapies. My research lab focuses this effort on the devastating and widespread disease Lassa fever, and has projects on malaria, cholera, and Ebola. I consider mentoring to be one of my most important roles and am committed to nurturing the careers of the post-docs, graduate students, and other trainees in my lab.

B. Positions and Honors.

Positions and Employment

1994-1997 Undergraduate Research Assistant for Professor David Bartel, Whitehead Institute for Biomedical Research, Cambridge, MA
1998-2000 Graduate Research Scientist for Professors Ryk Ward and Dominic Kwiatkowski, Oxford University, Oxford, UK
2000-2007 Graduate Research Scientist and Post-doctoral fellow for Professor Eric Lander, Broad Institute of MIT and Harvard (formerly Whitehead Institute for Biomedical Research), Cambridge, MA
2008-2012 Assistant Professor, Harvard University, Center for Systems Biology, Department of Organismic and Evolutionary Biology, Cambridge, MA
2008-2010 Associate Member, Broad Institute of MIT and Harvard, Cambridge, MA
2010-present Senior Associate Member, Broad Institute of MIT and Harvard, Cambridge, MA
2012-present Associate Professor, Harvard University, Center for Systems Biology, Department of Organismic and Evolutionary Biology, Cambridge, MA
2012-present Associate Professor, Harvard School of Public Health, Department of Immunology and Infectious Disease, Boston, MA

Other Experience and Professional Memberships

2012-present World Economic Forum, Young Global Leader, Global Agenda Council member for Personalized and Precision Medicine
2007-2012 National Academy of Science, Committee on Women in Science, Engineering and Medicine
1999-2004 MIT Corporation (Board of Trustees). Member of the Corporation. Member of Visiting Committees for Humanities (co-chair), Biological Engineering, and Libraries
2003-present Referee for peer-reviewed journals including Nature, Science, Cell, Genome Research, PLoS Biology, PLoS Genetics, New England Journal of Medicine, Human Molecular Genetics, Molecular Biology and Evolution, Nature Genetics, Nature Reviews Genetics, PLoS One, and American Journal of Human Genetics

Honors

1997-2000 Rhodes Scholarship

2001-2003	Paul and Daisy Soros Fellowship for New Americans
2004-2005	L'Oreal for Women in Science Fellowship
2004-2006	Damon Runyon Cancer Research Fellowship
2006-present	Burroughs Wellcome Career Award in Biomedical Sciences
2006	Harvard Medical School M.D. *summa cum laude*
2006	Science Spectrum Magazine Trailblazer
2007	Genome Technology Magazine, Tomorrow's PIs
2007	Seed Magazine Revolutionary Mind
2008	Packard Foundation Fellowship in Science and Engineering
2009	NIH Innovator Award
2011	PopTech Science Fellow
2011	Grinnell College Honorary Doctorate Degree
2012	World Economic Forum Young Global Leader
2012	Smithsonian American Ingenuity Award for Natural Science
2013	Massachusetts Academy of Sciences Fellow
2013	Ellis Island Medal of Honor
2013	National Geographic Emerging Explorer

C. Selected Peer-reviewed Publications (15)

1. **Sabeti PC**, Reich DE, Higgins JM, Levine HZ, Richter DJ, Schaffner SF, Gabriel SB, Platko JV, Patterson NJ, McDonald GJ, Ackerman HC, Campbell SJ, Altshuler D, Cooper R, Kwiatkowski D, Ward R, Lander ES. Detecting recent positive selection in the human genome from haplotype structure. *Nature.* 2002;419(6909):832-7. PMID: 12397357.
2. **Sabeti PC**, Walsh E, Schaffner SF, Varilly P, Fry B, Hutcheson HB, Cullen M, Mikkelsen TS, Roy J, Patterson N, Cooper R, Reich D, Altshuler D, O'Brien S, Lander ES. The case for selection at CCR5-Delta32. *PLoS Biology.* 2005;3(11):e378. PMID: 16248677.
3. **Sabeti PC**, Schaffner SF, Fry B, Lohmueller J, Varilly P, Shamovsky O, Palma A, Mikkelsen TS, Altshuler D, Lander ES. Positive natural selection in the human lineage. *Science*. 2006; 312(5780):1614-20. PMID: 16778047.
4. **Sabeti PC**, Varilly P, Fry B, Lohmueller J, Hostetter E, Cotsapas C, Xie X, Byrne EH, McCarroll SA, Gaudet R, Schaffner SF, Lander ES, and the International Haplotype Consortium. Genome-wide detection and characterization of positive selection in human populations. *Nature*. 2007;449(7164):913-8. PMID: 17943131.
5. **1000 Genomes Project Consortium**, Durbin RM, Abecasis GR, Altshuler DL, Auton A, Brooks LD, Durbin RM, Gibbs RA, Hurles ME, McVean GA. A map of human genome variation from population-scale sequencing. *Nature*. 2010;467(7319):1061-73. PMID: 20981092.
6. Altshuler DM, Gibbs RA, Peltonen L, Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, Peltonen L, Dermitzakis E, Bonnen PE, Altshuler DM, Gibbs RA, de Bakker PI, Deloukas P, Gabriel SB, Gwilliam R, Hunt S, Inouye M, Jia X, Palotie A, Parkin M, Whittaker P, Yu F, Chang K, Hawes A, Lewis LR, Ren Y, Wheeler D, Gibbs RA, Muzny DM, Barnes C, Darvishi K, Hurles M, Korn JM, Kristiansson K, Lee C, McCarrol SA, Nemesh J, Dermitzakis E, Keinan A, Montgomery SB, Pollack S, Price AL, Soranzo N, Bonnen PE, Gibbs RA, Gonzaga-Jauregui C, Keinan A, Price AL, Yu F, Anttila V, Brodeur W, Daly MJ, Leslie S, McVean G, Moutsianas L, Nguyen H, Schaffner SF, Zhang Q, Ghori MJ, McGinnis R, McLaren W, Pollack S, Price AL, Schaffner SF, Takeuchi F, Grossman SR, Shlyakhter I, Hostetter EB, **Sabeti PC**, Adebamowo CA, Foster MW, Gordon DR, Licinio J, Manca MC, Marshall PA, Matsuda I, Ngare D, Wang VO, Reddy D, Rotimi CN, Royal CD, Sharp RR, Zeng C, Brooks LD, McEwen JE. Integrating common and rare genetic variation in diverse human populations. *Nature.* 2010;467(7311):52-8. PMID: 20811451.
7. Grossman SR, Shlyakhter I, Karlsson EK, Byrne EH, Morales S, Frieden G, Hostetter E, Angelino E, Garber M, Zuk O, Lander ES, Schaffner SF, **Sabeti PC**. A composite of multiple signals distinguishes causal variants in regions of positive selection. *Science.* 2010;327(5967):883-6. PMID: 20056855.

8. Broadbent KM, Park D, Wolf AR, Van Tyne D, Sims JS, Ribacke U, Volkman S, Duraisingh M, Wirth D, **Sabeti PC**, Rinn JL. A global transcriptional analysis of Plasmodium falciparum malaria reveals a novel family of telomere-associated lncRNAs. *Genome Biology*. 2011;12(6):R56. PMID: 21689454.
9. Reshef DN, Reshef YA, Finucane HK, Grossman SR, McVean G, Turnbaugh PJ, Lander ES, Mitzenmacher M, **Sabeti PC**. Detecting novel associations in large data sets. *Science.* 2011;334(6062):1518-24. PMID: 22174245.
10. Andersen KG, Shylakhter I, Tabrizi S, Grossman SR, Happi CT, **Sabeti PC**. Genome-wide scans provide evidence for positive selection of genes implicated in Lassa fever. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*. 2012;367(1590):868-77. PMID: 22312054.
11. Grossman SR, Andersen KG, Shlyakhter I, Tabrizi S, Winnicki S, Yen A, Park DJ, Griesemer D, Karlsson EK, Wong SH, Cabili M, Adegbola RA, Bamezai RN, Hill AV, Vannberg FO, Rinn JL, Lander ES, Schaffner SF, **Sabeti PC**. Identifying recent adaptations in large-scale genomic data. *Cell*. 2013;152(4):703-13. PMID: 23415221.
12. Kamberov YG, Wang S, Tan J, Gerbault P, Wark A, Tan L, Yang Y, Li S, Tang K, Chen H, Powell A, Itan Y, Fuller D, Lohmueller J, Mao J, Schachar A, Paymer M, Hostetter E, Byrne E, Burnett M, McMahon AP, Thomas MG, Lieberman DE, Jin L, Tabin CJ, Morgan BA, **Sabeti PC**. Modeling recent human evolution in mice by expression of a selected EDAR variant. *Cell*. 2013;152(4):691-702. PMID: 23415220.
13. Farhat MR, Shapiro BJ, Kieser KJ, Sultana R, Jacobson KR, Victor TC, Warren RM, Streicher EM, Calver A, Sloutsky A, Kaur D, Posey JE, Plikaytis B, Oggioni MR, Gardy JL, Johnston JC, Rodrigues M, Tang PK, Kato-Maeda M, Borowsky ML, Muddukrishna B, Kreiswirth BN, Kurepina N, Galagan J, Gagneux S, Birren B, Rubin EJ, Lander ES, **Sabeti PC**, Murray M. Genomic analysis identifies targets of convergent positive selection in drug-resistant Mycobacterium tuberculosis. *Nature genetics*. 2013;45(10):1183-9. PMID: 23995135.
14. Karlsson EK, Harris JB, Tabrizi S, Rahman A, Shlyakhter I, Patterson N, O'Dushlaine C, Schaffner SF, Gupta S, Chowdhury F, Sheikh A, Shin OS, Ellis C, Becker CE, Stuart LM, Calderwood SB, Ryan ET, Qadri F, **Sabeti PC**, Larocque RC. Natural selection in a bangladeshi population from the cholera-endemic ganges river delta. *Science Translational Medicine*. 2013;5(192):192ra86. PMID: 23825302.
15. 16. Shaffer JG, Grant D Sand Schieffelin JS *et al.* Lassa fever in post-conflict Sierra Leone. PLoS Neglected Tropical Diseases 2014, in press.

D. Research Support

CURRENT SUPPORT

NIH 1R01AI103055-01A1 (Jason Harris) 12/1/13-11/30/14
NIH (Harvard Subcontract)
Innate and Early B Cell Responses to Vibrio Cholerae
The goal of our sub-aim is to evaluate the association between early immunologic responses to *V. cholerae* and the subsequent development of adaptive immunity.
Role: Co-investigator

Private Source **Dyann Wirth, Eric Lander)** 7/1/2009 – 11/30/2015
(Harvard Subcontract)
Genomic-Based Diagnostics for Elimination and Eradication of Plasmodium
The goal of this project is to use high-throughput and field-deployable genotyping tools of *P. falciparum* parasite populations to enhance current efforts for surveillance and control of malaria.
Role: PI of subcontract

Private Source **(Pardis Sabeti)** 9/1/2008 – 1/20/2014
Private Source (Harvard)
The goals of this project are (1) to develop computational tools to localize and characterize signals of natural selection identified in humans, and (2) to carry out biological follow up of top candidates for natural selection.
Role: PI

NIH MIDAS Centers of Excellence (Marc Lipsitch) 9/1/2009 – 8/31/2014
NIH (Harvard subcontract)
The goal of our sub-aim is to create, in collaboration with potential users from public health agencies, "dashboard" software for visualization and analysis of data from emerging epidemics of novel diseases.
Role: PI of subcontract

NIAID HHSN272200900049C (James Robinson) 9/30/2009 – 9/29/2014
NIAID (Harvard and Broad Subcontracts)
Roles of protective or pathogenic B cell epitopes in human Lassa Fever
The goal of this program is to identify novel B cell epitopes on Lassa virus proteins and elucidate novel mechanisms of antibody-mediated protection or pathogenesis in humans. The sub-award aims to determine Lassa virus sequence diversity and its impact on recognition of B cell epitopes by Lassa fever patients.
Role: PI of subcontract

NIH 1DP2OD006514-01 (Pardis Sabeti) 1/1/2010 – 8/31/2014
NIH (Harvard)
Host and Pathogen Evolution in Lassa Fever
The goals of this project are to: (1) Develop computational and experimental methods to investigate natural selection in humans 1) Identify host and pathogen genetic determinants of Lassa fever susceptibility.
Role: PI

NIAID HHSN272201000022C (Pardis Sabeti) 9/30/2010 – 9/29/2015
NIAID (Broad Contract and Harvard Subcontract)
Host Genetic Factors in Resistance to Lassa Hemorrhagic Fever
The goal of this project is to replicate strong signals of natural selection found in Yorubans at genes critical for infection with Lassa virus, carry out a genome-wide association study for Lassa fever susceptibility, and to localize and characterize the key functional mutations identified.
Role: PI of contract and subcontract

COMPLETED SUPPORT

W81XWH-11-1-0141 (Lisa Hensley) 12/30/10 – 12/29/12
DOD (Harvard Subcontract)
Microarray processing and analysis of biological samples from non-human primate models of viral infections and human clinical samples.
The project goals are to: (1) identify biomarkers from non-human primate samples, (2) assay human clinical specimens to generate data for biomarker discovery, (3) assay the human clinical samples for viral genome sequences, and (4) validate human biomarkers by testing in human populations. Role: PI of subcontract

Private Source 1/1/12 – 12/31/12
(Broad)
Pathogen-independent platform for sequencing from clinical samples using next generation technologies.
The project goal is to develop molecular methods to sequence microbial genomic material directly from clinical samples, creating a pathogen-independent platform that can be scaled up into a high-throughput, high-resolution and adaptive platform. This platform will allow novel virus/bacteria/fungus discovery, as well as profiling of the pathogen population that may exist during infection. Role: PI

Private Source **Pardis Sabeti)** 1/1/08 – 8/31/13
Private Source (Harvard)
Evolutionary genomics and its application to human disease
The project goal is to develop and apply computational methods to detect evidence of natural selection in genetic variation data in humans. Role: PI

BIOGRAPHICAL SKETCH

NAME Erica Ollmann Saphire	POSITION TITLE Professor
eRA COMMONS USER NAME eRA Commons User Name	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Rice University, Houston, TX	B.A.	1989-1993	Ecol&Evol; Biochem.
The Scripps Research Institute, La Jolla, CA	Ph.D.	1994-2000	Structural Biology
The Scripps Research Institute, La Jolla, CA	Post-doc	2000-2002	Structural Immunology

A. Personal Statement

The Ollmann Saphire lab determines the structural basis of viral pathogenesis, with a special emphasis on hemorrhagic fever viruses. We have determined all available structures of the oligomeric, prefusion filovirus GP in cleaved and uncleaved forms. We have also determined multiple crystal structure of arenavirus nucleoproteins, matrices, and glycoproteins. These structures have proposed new biological functions and structural rearrangements that could be targeted for antiviral design. In this effort, we have amassed hundreds of different constructs to express the antigens, mapped multiple antibody epitopes, and have provided pioneering new insights into how the very few gene products of these viruses combine to cause pathogenesis. The driving force of our structural work is the opportunity to propose and develop new biological hypotheses about viral attachment, entry, and neutralization. Indeed, our structures have allowed us to propose the receptor-binding sites, consequences of cleavage, and conformational changes required for fusion with target cell endosomes and have opened multiple downstream lines of inquiry.

B. Positions

2003 - 2008	Assistant Professor, Immunology, TSRI: *Viral Hemm. Fever Pathogenesis.*
2008 -	Member, Skaggs Institute for Chemical Biology, TSRI
2008 - 2012	Associate Professor, Dept. of Immunology and Microbial Science, TSRI
2012 - present	Professor, Dept. of Immunology and Microbial Science, TSRI

Honors

2003	Sidhu Award, most outstanding contribution to the field of diffraction by a person within 5 years of the Ph.D.
2003	Ellison Medical Foundation New Initiatives Award in Global Infectious Disease
2003 - 2008	Burroughs Wellcome Career Award in the Biomedical Sciences
2006	ICAAC Young Investigator Award, American Society for Microbiology
2009	Presidential Early Career Award in Science and Engineering (PECASE), The White House Office of Science and Technology Policy and NIH/NIAID
2009 - 2014	Burroughs Wellcome Investigator in the Pathogenesis of Infectious Disease
20111 -	Co-Director, Center of Excellence, The Global Virus Network
2012 -	Editorial Board, Annual Review of Virology
2013	NIH Director's Lecture, NIH
2013 -	Director, Viral Hemorrhagic Fever Immunotherapeutic Consortium
2013 -	Scientific Leadership Board, Global Virus Network
2014	Fellow, American Academy of Microbiology

C. Selected Peer-reviewed Publications

1. Bornholdt, Z. A., T. Noda, D. M. Abelson, P. Halfmann, M. R. Wood, Y. Kawaoka, and **E. O. Saphire.** 2013. Structural basis for ebolavirus matrix assembly and budding; protein plasticity promotes multiple functions. Cell,
2. Zhang, A. P., Z. A. Bornholdt, T. Liu, D. M. Abelson, D. E. Lee, S. Li, V. L. Woods, Jr., and **E. O. Saphire**. 2012. The ebola virus interferon antagonist VP24 directly binds STAT1 and has a novel, pyramidal fold. PLoS Pathog 8:e1002550. PMC3285596
3. Olal, D., A. I. Kuehne, S. Bale, P. Halfmann, T. Hashiguchi, M. L. Fusco, J. E. Lee, L. B. King, Y. Kawaoka, J. M. Dye, Jr., and **E. O. Saphire**. 2012. Structure of an antibody in complex with its mucin domain linear epitope that is protective against Ebola virus. J Virol 86:2809-2816. PMC3302272
4. Hastie, K. M., L. B. King, M. A. Zandonatti, and **E. O. Saphire**. 2012. Structural Basis for the dsRNA Specificity of the Lassa Virus NP Exonuclease. PLoS One 7:e44211. PMC3429428
5. Bale, S., J. P. Julien, Z. A. Bornholdt, C. R. Kimberlin, P. Halfmann, M. A. Zandonatti, J. Kunert, G. J. Kroon, Y. Kawaoka, I. J. MacRae, I. A. Wilson, and **E. O. Saphire**. 2012. Marburg virus VP35 can both fully coat the backbone and cap the ends of dsRNA for interferon antagonism. PLoS Pathog 8:e1002916. PMC3441732
6. Hastie, K. M., T. Liu, S. Li, L. B. King, N. Ngo, M. A. Zandonatti, V. L. Woods, Jr., J. C. de la Torre, and **E. O. Saphire**. 2011. Crystal structure of the Lassa virus nucleoprotein-RNA complex reveals a gating mechanism for RNA binding. Proc Natl Acad Sci U S A 108:19365-19370. PMC3228486
7. Hastie, K. M., C. R. Kimberlin, M. A. Zandonatti, I. J. Macrae, and **E. O. Saphire**. 2011. Structure of the Lassa virus nucleoprotein reveals a dsRNA-specific 3' to 5' exonuclease activity essential for immune suppression. Proc Natl Acad Sci U S A. PMC3038715
8. Dias, J. M., A. I. Kuehne, D. M. Abelson, S. Bale, A. C. Wong, P. Halfmann, M. A. Muhammad, M. L. Fusco, S. E. Zak, E. Kang, Y. Kawaoka, K. Chandran, J. M. Dye, and **E. O. Saphire**. 2011. A shared structural solution for neutralizing ebolaviruses. Nat Struct Mol Biol 18:1424-1427. PMC3230659
9. Bale, S., T. Liu, S. Li, Y. Wang, D. Abelson, M. Fusco, V. L. Woods, Jr., and **E. O. Saphire**. 2011. Ebola virus glycoprotein needs an additional trigger, beyond proteolytic priming for membrane fusion. PLoS Negl Trop Dis 5:e1395. PMC3216919
10. Kimberlin, C. R., Z. A. Bornholdt, S. Li, V. L. Woods, Jr., I. J. MacRae, and **E. O. Saphire**. 2010. Ebolavirus VP35 uses a bimodal strategy to bind dsRNA for innate immune suppression. Proc Natl Acad Sci U S A 107:314-319. PMC2806767
11. Lee, J. E., M. L. Fusco, and **E. O. Saphire**. 2009. An efficient platform for screening expression and crystallization of glycoproteins produced in human cells. Nat Protoc 4:592-604. 2911120
12. Lee, J. E., M. L. Fusco, D. M. Abelson, A. J. Hessell, D. R. Burton, and **E. O. Saphire**. 2009. Techniques and tactics used in determining the structure of the trimeric ebolavirus glycoprotein. Acta Crystallogr D Biol Crystallogr 65:1162-1180. PMC2777170
13. Lee, J. E., A. Kuehne, D. M. Abelson, M. L. Fusco, M. K. Hart, and **E. O. Saphire**. 2008. Complex of a protective antibody with its Ebola virus GP peptide epitope: unusual features of a V lambda x light chain. J Mol Biol 375:202-216. PMC2173910
14. Lee, J. E., M. L. Fusco, A. J. Hessell, W. B. Oswald, D. R. Burton, and **E. O. Saphire**. 2008. Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. Nature 454:177-182. PMC2700032
16. Shaffer JG, Grant D Sand Schieffelin JS *et al.* Lassa fever in post-conflict Sierra Leone. PLoS Neglected Tropical Diseases 2014, in press.

D. Research Support

Ongoing

R01 AI067927 Saphire (PI) 07/01/06 - 06/30/14
NIH/NIAID

Ebola Viral Glycoproteins: Structural Analysis

The specific aims of this project are to determine structures of the Ebola virus GP and antibodies directed against it.

Role: PI

R01 AI081982 Saphire (PI) 12/01/09 - 11/30/14
NIH/NIAID
Structure Driven Analysis of Ebola Virus Receptor Binding Site
The specific aims of this project are to understand the location and composition of the receptor-binding site, the degree to which it is masked prior to enzymatic cleavage, and the nature and trigger of conformational changes in GP2 associated with fusion.
Role: PI

R01 AI089498 Saphire (PI) 07/01/10 - 06/30/15
NIH/NIAID
Analysis of Immunotherapeutics to Native Marbug virus epitopes
The specific aims of this project are to raise a large panel of mAbs against Marburg virus, and characterize them structurally and functionally.
Role: PI

AI2008031 Garry (PI) 08/17/09 - 08/16/14
NIH/NIAID
B Cell Epitope Discovery and Mechanisms of Antibody Protection
Project to understand antibody epitopes on lassa virus glycoprotein. A large panel of human antibodies against Lassa virus will be raised by the prime institution, Here, these antibodies will be analyzed structurally in complex with Lassa virus antigens. Role: Contributor
Role: Co-PI

R01 AI101436-02 Chandran (PI) 05/29/12 – 04/30/17
NIH
Endosomal receptor-mediated entry of filoviruses into host cells
Subcontract for co-crystallization of Ebola virus GP in complex with NPC1 receptor. Project overseen by experienced postdoc, Staff Scientist, and two lab managers.
Role: Consortium PI

Private Source Saphire (PI) 10/1/09 - 09/30/12
Structural Analysis of *Ebolavirus* nucleocapsid assembly
Role: PI

Private Source Saphire (PI) 11/01/09 - 10/31/15
Investigators in the Pathogenesis of Infectious Disease
Role: PI

Completed

Private Source Award in the Biomedical Sciences 09/15/03 - 09/14/09
Career Award for promising young faculty; No specific aims associated with this project.
Role: PI

U01 AI070530 Saphire (PI) 08/01/06 - 07/31/11
NIH/NIAID
Immunotherapeutics vs. Ebola Sudan: Development, Structural & Functional Analysis
The specific aims of this project were to raise monoclonal antibodies against the Sudan subtype of the Ebola virus, map their epitopes and determine which are protective in animal models. Role: PI
Role: PI

U01 AI082156 Saphire (PI) 09/01/09 - 08/31/11
NIH
Immunotherapeutics Against Marburg: Development, Structural & Functional Analysis

The specific aims are to engineer MARV GP for crystallization and immunization.
Role: PI

R43 AI088843 Geske (PI) 04/01/10 - 03/31/12
NIH/NIAID
Recombinant Filovirus Diagnostics: Production and analysis of recombinant *ebolavirus* and *marburgvirus* GP for use in lateral flow point-of-care immunodiagnostics.
Role: Co-PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
John S. Schieffelin	Assistant Professor of Clinical Medicine Departments of Pediatrics and Internal Medicine Sections of Infectious Diseases
eRA COMMONS USER NAME eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Cornell University, Ithaca, NY	AB.	1988-92	Biology
Tulane School of Public Health and Tropical Medicine, New Orleans, LA	MSPH	1993-95	Parasitology
Tulane School of Medicine, New Orleans, LA	M.D.	1997-2001	Medicine

A. Personal Statement

After completing a medical residency in Internal Medicine and Pediatrics, I began a combined fellowship in Adult and Pediatric Infectious Disease at Tulane University and Louisiana State University. During this time, I began my research investigating the human antibody repertoire in response to dengue virus infection. Currently my research program explores the humoral immune response to flaviviruses and Lassa fever virus. The role of specific viral epitopes and immunoglobulin subclasses in many infectious diseases are unknown. Using novel screening techniques, I will continue to generate a unique set of human monoclonal antibody reagents and explore the response of the host humoral immune system to viral infection. The antibodies are also used to incease our understanding of the pathogenesis of disease as well as to develop next generation diagnostic tests and immunotherapeutics. I have the expertise, leadership and motivation necessary to successfully carry out the proposed work. I have a broad background in clinical infectious disease, international clinical research, immunology and virology with specific training and expertise in key research areas for this application. This research has allowed me to lay the groundwork for the proposed research by developing necessary laboratory infrastructure. I have successfully administered the projects (e.g. staffing, human subjects protections, budget), collaborated with a diversity of other researchers, and produced several peer-reviewed publications with several more in preparation. In summary, I have a demonstrated record of successful and productive research projects in an area of high relevance to infectious disease and global health. In addition, my expertise and relevant administrative experience have prepared me for the proposed project.

B. Positions and Honors

2001-2005 Medical Resident in Internal Medicine and Pediatrics, Tulane University School of Medicine

2005-2009 Postdoctoral Fellow in Adult and Pediatric Infectious Diseases, Louisiana State University School of Medicine and Tulane University School of Medicine

2009- Assistant Professor of Clinical Medicine, Departments of Internal Medicine and Pediatrics, Sections of Infectious Disease

Other Professional Activities

1994-1995 Peace Corps Volunteer, Comoros Islands

2001- Member, American Academy of Pediatrics

2003- Member, American College of Physicians

2005- Member, Infectious Disease Society of America

C. Selected Publications

1. **Schieffelin JS**, Costin JM, Nicholson CO et al. Neutralizing and non-neutralizing monoclonal antibodies against dengue virus E protein derived from a naturally infected patient. Virol J. 2010 Feb 4;7(1):28. PMID: 20132551

2. Grove JN, Branco LM, Boisen ML, Muncy IJ, Henderson LA, **Schieffelin JS**, Robinson JE, Bangura JJ, Fonnie M, Schoepp RJ, Hensley LE, Seisay A, Fair JN, Garry RF. Capacity building permitting comprehensive monitoring of a severe case of Lassa hemorrhagic fever in Sierra Leone with a positive outcome: case report. Virol J. 2011 Jun 20;8(1):314. PMID: 21689444

3. Branco LM, Boisen ML, Andersen KG, Grove JN, Moses LM, Muncy IJ, Henderson LA, **Schieffelin JS**, Robinson JE, Bangura JJ, Grant DS, Raabe VN, Fonnie M, Sabeti PC, Garry RF.Lassa Hemorrhagic Fever in a Late Term Pregnancy from Northern Sierra Leone with a Positive Maternal Outcome: Case Report. Virol J. 2011 Aug 15;8(1):404. PMID: 21843352

4. Costin JM, Zaitseva E, Kahle KM, Nicholson CO, Rowe DK, Graham AS, Bazzone LE, Hogancamp G, Sierra MF, Fong RH, Yang ST, Lin L, Robinson JE, Doranz BJ, Chernomordik LV, Michael SF, **Schieffelin JS**, Isern S. Mechanistic study of broadly neutralizing monoclonal anitbodies against dengue virus that target the fusion loop. J Virol. 2012 Oct 17. PMID 23077306

5. Bond N, **Schieffelin JS**, Moses L, Bennett A, Bausch D. A historical look at the first reported cases of lassa fever: IgG antibodies 40 years after acute infection. Amer J Tropic Med Hyg. PMID 23390223

6. **Schieffelin JS**, Williams PL, Djokic D, Anderson JP, Nachman S, Oleske JM, Seage GR, Van Dyke RB. Central nervous system vasculopathy in Human Immunodeficiency Virus-infected children enrolled in the Pediatric AIDS Clinical Trials Group 219/219C study. J Pediat Infect Dis. 2013;2(1):50.

7. **Schieffelin JS**, Torrellas M, Lartchenko S, Gill F, Garcia-Diaz J, McGoey R. How natural disasters change natural patterns: coccidioidomycosis in New Orleans. J La State Med Soc. 2013; 165(3):145. PMID 24015428

8. **Schieffelin JS**, Garcia-Diaz JB, Loss GE, Beckman EN, Keller RA, Staffeld-Coit C, Garces JC, Pankey GA. Phaeohyphmycosis Fungal Infections in Solid Organ Transplant Recipients: Clinical Presentation, Pathology and Treatment. Transplant Infect Dis. *In Press*.

9. Shaffer JG, Grant DS, **Schieffelin JS**, Boisen ML, Goba A, Hartnett JN, Levy DC, Yenni RE, Moses LM, Fullah M, Momoh M, Fonnie M, Fonnie R, Kanneh L, Koroma VJ, Kargbo K, Ottomassathien D, Muncy IJ, Jones AB, Illick MM, Kulakosky PC, Haislip AM, Bishop CM, Elliot DH, Brown BL, Zhu H, Hastie KM, Andersen KG, Gire SK, Tabrizi S, Tariyal R, Stremlau M, Matschiner A, Sampey DB, Spence JS, Cross RW, Geisbert JB, Folarin OA, Happi CT, Pitts KR, Geske FJ, Geisbert TW, Saphire EO, Robinson JE, Wilson RB, Sabeti PC, Henderson LA, Khan SH, Bausch DG, Branco LM, Garry RF. Lassa Fever in Post-Conflict Sierra Leone.PLOS NTD. *Accepted.*

D. Research Support:
Ongoing Research Support

P20RR021970-06 Ochoa (Overall PI) Schieffelin (Project 5 PI) 8/1/10-6/30/15
Mentoring Translational Researchers in Louisiana
Source: NIH-NCRR
The goal of this grant is to assist young physician-scientists in developing research projects and collecting adequate preliminary data so that they can compete successfully for independent funding in the near future. The project under study is titled "Identification of Protective and Pathogenic Human B Cell Epitopes in Dengue Virus." The goal of this study is to develop anti-Dengue Virus human monoclonal antibodies and to characterize their activity and function in terms of neutralization and enhancement. Analysis of the typical human antibody response to natural infection will provide a better understanding of protective and pathogenic responses and will identify ideal vaccine targets as well as possible therapeutics.
Role: Project Leader % effort

BAA-NIAID-DAIT-NIHAI2008031 Robinson (PI) 9/1/10-8/30/14
Roles of Protective and Pathogenic B Cell epitopes in Human Lassa Fever

Source: National Institutes of Health
Dr. Schieffelin's role in the large multi-center project is to generate human monoclonal antibodies against the Lassa Virus from blood samples collected in the field. This effort is critical to understanding the role of anti-Lassa virus antibodies in the pathogenesis of Lassa fever and to identify immunogenic, protective vaccine targets.
Role: Co-Investigator % effort

1U01AI082119-03 Garry (PI) 4/01/09-3/31/14
Pre-Clinical development of recombinant antigen diagnostics for Lassa Fever
Source: National Institutes of Health
The goal of this project is to develop, validate and market rapid diagnostic tests for Lassa Hemorrhagic Fever. Dr. Schieffelin's role as Clinical Director involves developing the clinical testing of the diagnostic tests in Sierra Leone.
Role: Co-Investigator % effort

NIAID-DAIT-NIH2009061 Garry (PI) 6/01/10-5/31/16
Host Genetic Factors in Resistance to Lassa Hemorrhagic Fever
Source: National Institutes of Health
Dr. Schieffelin's role in this international, multi-center study is to over see all human subjects research.
Role: Co-Investigator % effort

Completed Research Support
Postdoctoral Research Award Robinson (PI) 7/1/08-6/30/10
Neutralization and Enhancement of Dengue Virus by Human Monoclonal Antibodies
Source: South Louisiana Institute for Infectious Disease Research Postdoctoral Support Award.
This was a mentored grant designed to allow the recipient to collect pilot data necessary for inclusion in federal competitive grants.
Role: Co-Investigator % effort

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert F.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Shaffer, Jeffrey, George	Research Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of New Orleans, New Orleans, LA	B.S.	1999	Mathematics
University of New Orleans, New Orleans, LA	M.S.	2001	Mathematics
Tulane University, New Orleans, LA	Ph.D.	2007	Biostatistics
Tulane University, New Orleans, LA	Postdoc	2010	Biostatistics

A. Personal Statement

My research interests are focused on the statistical analysis of health and clinical data, specifically for febrile illnesses in West Africa, environmental exposures, breast and prostate cancers, and infant mortality. My areas of expertise include statistical programming, experimental design, population sampling, sample size and statistical power assessment, geographic information systems (GIS), spatial data analysis, hierarchical modeling, time trend analysis, survey design and analysis, and mobile and online surveys. My current research effort involves managing and analyzing clinical and laboratory data on Lassa fever (an acute febrile disease found in West Africa) as part of a large consortium to develop a rapid diagnostic test for Lassa fever. I regularly teach graduate-level courses in geographic information systems, introductory biostatistics, and SAS statistical programming.

B. Positions and Honors

Positions and Employment

1998 – 2001 Instructor and Graduate Teaching Assistant, Department of Mathematics, University of New Orleans, New Orleans, LA
2000 – 2001 Textbook Editor, JRL Enterprises, Inc., New Orleans, LA
2001 – 2007 Instructor and Graduate Research Assistant, Department of Biostatistics, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA
2007 – 2010 Postdoctoral Fellow, Department of Biostatistics, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA
2010 – present Research Assistant Professor, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA

Professional Memberships

1998 – present Member, Mathematical Association of America
2004 – present Member, American Statistical Association
2005 – present Member, American Red Cross
2007 – present Member, Tulane Alumni Association
2007 – present Vice president, Delta Omega Honorary Society in Public Health

Honors

2002 “Who’s Who Among Students in American Universities & Colleges,” Tulane University, New Orleans, LA

2005 Excellence in Biostatistics Award, Tulane University, New Orleans, LA
2007 Dean's Scholastic Award, Tulane University, New Orleans, LA
2009 Recipient of Undergraduate Public Health Studies Research and Teaching Award, Tulane University, New Orleans, LA

C. Selected Peer-reviewed Publications

1. Mather, F., White, L., Langlois, E., Shorter, C., Swalm, C., Shaffer, J., & Hartley, W. (2004). Statistical methods for linking health, exposure and hazards. *Environmental Health Perspectives*, 112(14), 1440-1445.

2. Mather, F., Chen, V., Morgan, L., Correa, C., Shaffer, J., Srivastav, S., Rice, J., Blount, G., Swalm, C., Wu, X., & Scribner, R. (2006). Hierarchical modeling and other spatial analyses in prostate cancer incidence data. *American Journal of Preventive Medi*cine, 30(2), S88-100.

3. Shaffer, J. & Srivastav, S. (2009). A simple technique for constructing optimal complete diallel cross designs. *Statistics & Probability Letters*, 79(9), 1181-1185.

4. Branco, L., Grove, J., Boisen, M., Shaffer, J., Goba, A., Fullah, M., Momoh, M., Momoh, M., Grant, D., & Garry, R. (2011). Emerging trends in Lassa fever: redefining the role of immunoglobulin M and inflammation in diagnosing acute infection. *Virology Journal*, 8:478.

5. Shaffer, J., Grant., D., Schieffelin, J., Boisen, M., Goba, A., Hartnett, J., … Garry, R. (in press). Lassa fever in post-conflict Sierra Leone. *PLOS Neglected Tropical Diseases*.

D. Research Support

Ongoing Research Support

5U19AI089696-04 Krogstad (PI) 8/1/13 – present
NIH
POPULATION-BASED APPROACH TO MALARIA RESEARCH AND CONTROL

This work compares four sites in West Africa with regard to intensity and prevalence of malaria infection, vector mosquito population dynamics, including insecticide resistance, disease pathogenesis with a focus on host factors and the immune response and the emergence and spread of drug resistance. Both the ecological environment and the parasite are known to modulate the disease outcome on an individual and population level, and this project seeks to understand the relationship between these external factors and the underlying genetic determinants in the host, parasite and vector populations. This project is a component of the International Centers for Excellence for Malaria Research (ICEMR).
Role: Co-Investigator, Data Management and Biostatistics Core Leader

272200900049C-0-0-1 Robinson (PI) 4/1/10 – present
NIH
Roles of Protective or Pathogenic B Cell Epitopes in Human Lassa Fever
This research project applies microbiology techniques to identify novel B cell epitopes from Lassa fever virus blood samples in an effort to elucidate mechanisms that are important for human protection or immunity from the virus. The study subjects include rodents and humans in West Africa. The project team consists of researchers at Tulane University, Harvard University, and several biomedical companies.
Role: Co-Investigator

Program Director/Principal Investigator (Last, First, Middle): Garry, Robert F.

Completed Research Support

3D43TW007784-04S1 Buekens (PI) 9/1/09 – 8/31/11
NIH
Training for Evidence-Based Health Care Research, Argentina
Part of the initiative for Tulane's International Clinical, Operational, and Health Services Research and Training Award (ICOHRTA), the project involved developing an audiovisual distance learning course on the use of mobile technologies for conducting PDA-based surveys. Course topics included the complementary use of GPS and PDA devices, PDA-based electronic form design, and mobile data management methods.

Private Source Mather (PI) 7/1/08 – 6/10/10
Expanding GIS Research and Education Opportunities at Tulane University
The project was an interdisciplinary effort to facilitate diverse GIS-related research collaborations and thereby increase extramural funding at Tulane University. Funds for this project were used to establish a contemporary GIS computer teaching laboratory and foster GIS-related communications among faculty and students via a host of online surveys and round table discussions.
Role: Co-Investigator

5D43TW007000 Mather (PI) 6/1/04 – 5/31/09
FIC
International Training for Global Health in Mali
This grant provided long, medium, and short-term informatics training to the employees of research, academic, and governmental institutions in Mali. The ultimate goal was to develop a Center of Excellence in informatics training for global health in West Africa.
Role: Co-Investigator

PA#02180 White (PI) 9/30/02 – 9/29/05
CDC
Center for Excellence in Environmental Health Tracking
The goal of this project was to assist in the development of the National Environmental Public Health Tracking Network (EPHTN) to further the understanding of the relationship between the environment and health outcomes.
Role: Co-Investigator

S1234-20 Mather (PI) 10/1/00 – 9/30/03 (NCE to 9/30/04)
CDC
Geographic Information Systems (GIS) and Prostate Cancer
This project involved identifying factors resulting in county-level prostate cancer incidence disparities in the state of Louisiana.
Role: Co-Investigator

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	INTRO_ICIDR_Specific_Aims.pdf
3. Research Strategy*	ICIDR_INTRO_final.pdf
4. Progress Report Publication List	Progress_Report_Publication_List.pdf
Human Subjects Sections	
5. Protection of Human Subjects	Overall_-_Human_Subjects.pdf
6. Inclusion of Women and Minorities	Overall_-_Women.pdf
7. Inclusion of Children	Overall_-_Children.pdf
Other Research Plan Sections	
8. Vertebrate Animals	Overall_Vert_Animals.pdf
9. Select Agent Research	Admin_Core_manage_Select_Agent_Research.pdf
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	Admin_Core_manage_Consortium_Agreement.pdf
12. Letters of Support	LoS_in_Admin_Core.pdf
13. Resource Sharing Plan(s)	Admin_Core_manage_Res_Sharing.pdf
Appendix (if applicable)	
14. Appendix	Overall_Multiple_PI.pdf

International Collaboration in Infectious Disease Research on Lassa fever (Lassa ICIDR)

SPECIFIC AIMS (OVERALL)

Lassa fever is an acute and often-fatal hemorrhagic disease caused by Lassa virus (LASV), an arenavirus. Lassa fever is a zoonotic infection, transmitted to humans by *Mastomys natalensis* (the multimammate "rat"). The highest incidence of Lassa fever in the world is in the Eastern Province of Sierra Leone. Signs and symptoms of LF, which occur 1-3 weeks after virus exposure, are highly variable, and can include fever, facial swelling, conjunctival injection, and vomiting. Frank bleeding occurs in less than a third of cases, but confers a poor prognosis. Signs and symptoms in LF patients who survive begin to subside 2-3 weeks after onset, but full recovery can require many months or longer.

Kenema Government Hospital (KGH) was an important site for Lassa fever clinical and laboratory research throughout the 1970s and 1980s. The violent civil conflict in Sierra Leone from 1991 to 2002, sometimes referred to as the Blood Diamonds War, forced suspension of Lassa fever research at KGH in 1993. Following cessation of hostilities, our consortium of Lassa fever researchers and other partners began rebuilding the scientific infrastructure at KGH, with a major focus of the development of improved laboratory diagnosis for Lassa fever. There is no vaccine for LF and the efficacy of the antiviral drug ribavirin in treating LF remains a matter of controversy. There is an urgent need to develop epidemiological and clinical measures to combat the public health challenges poised by Lassa fever in Sierra Leone and across West Africa. Promising next-generation Lassa fever diagnostic immunoassays, including rapid tests, require further development as epidemiological and clinical management tools.

Lassa ICIDR overall hypothesis: Further development of research capacity, with emphasis on training Sierra Leonean staff, will permit the KGH Lassa fever research program to emerge as an exceptional resource for human clinical trials of NIAID's promising portfolio of Lassa fever diagnostics, therapeutics, immunotherapeutics and vaccines.

Overall Specific Aims (listed in priority order):
Aim 1. Further enhance and utilize the symmetrical and highly productive partnership developed over the last decade between Tulane University and the Lassa fever program at KGH.

Aim 2. Promote the development of laboratory and clinical research capacity at KGH, with a particular emphasis on training Sierra Leonean staff.

Aim 3. Encourage future collaborative relationships with other research groups leading to improvements in detection, prevention, amelioration, and treatment of Lassa fever in the subregion.

Research question(s) to be addressed in the Lassa ICIDR Projects include:
Project 1: Can second generation Lassa fever recombinant immunoassays be used effectively as point-of-care diagnostics and surveillance tools for Lassa fever.

Project 2: What are the clinical and virological determinants of Lassa Fever outcome and is it possible to identify biomarkers of LASV infection and Lassa Fever outcome.

Through the proposed research we will acquire new information regarding the natural history of Lassa fever and the demographic distribution of people exposed to LASV using second generation Lassa fever recombinant immunoassays. We will also elucidate risk factors for acquiring serious or fatal LASV infection. This new information will guide evidence-based investments for public health programming and policy. We will also define biomarkers of Lassa fever pathobiology through the course of illness. Identification of these factors could lead to evidence-based approaches to reduce mortality from Lassa fever. We also propose to take a major step forward to confront the challenges of Lassa fever, namely developing the capacity to perform human clinical trials.

International Collaboration in Infectious Disease Research on Lassa fever (Lassa ICIDR)

OVERVIEW

Lassa fever is of significant public health importance to Sierra Leone. Unlike other severe viral hemorrhagic fevers (VHFs) that cause sporadic outbreaks, Lassa fever is endemic in Sierra Leone with cases presenting year round. While ten of thirteen Districts in Sierra Leone have recently reported Lassa fever cases, Kenema District in the Eastern Province has the world's highest Lassa fever incidence. Kenema Government Hospital (KGH) was an important site for Lassa fever clinical and laboratory research throughout the 1970s and 1980s. The violent civil conflict in Sierra Leone from 1991 to 2002, sometimes referred to as the Blood Diamonds War, forced suspension of Lassa fever research at KGH in 1993. Following cessation of hostilities, our consortium of Lassa fever researchers and other partners, including the Sierra Leone Ministry of Heath and Sanitation (MOHS), began rebuilding the scientific infrastructure at KGH, with a major focus of the development of improved laboratory diagnosis for Lassa fever. NIAID has provided the majority of support (financially and scientifically) for this effort largely through cooperative agreements and interactive contracts. The Lassa fever Program at KGH now provides diagnostic services and clinical care for over 500 suspected cases per year. The Lassa ICICR will further enhance and utilize the symmetrical and highly productive partnership developed over the last decade between Tulane University and the Lassa fever program at KGH. This is one of the only opportunities in the world to investigate the epidemiology and natural history of a severe VHF. As required by RFA-AI-14-002 International Collaborations in Infectious Diseases Research (U19), none of the problems studied can be addressed in developed countries.

There is an urgent need to develop epidemiological and clinical measures to combat the public health challenges poised by Lassa fever in Sierra Leone and across West Africa. Promising next-generation Lassa fever diagnostic immunoassays, including rapid tests, require further development as epidemiological and clinical management tools. There is no approved Lassa fever therapeutic or vaccine. Although the antiviral drug ribavirin can be beneficial, it must be administered at an early stage of infection to successfully alter disease outcome, thereby limiting its utility. The KGH Lassa fever research program is now poised to emerge as an exceptional resource for human clinical trials of a portfolio of Lassa fever diagnostics, therapeutics, immunotherapeutics and vaccines, now in advanced stages of development through NIAID supported research projects. These advances hold great promise for controlling, treating or preventing Lassa fever. The promise of these new technologies can only be realized if a program of training and capacity building is successful at KGH. Therefore, as an over-riding theme, the Lassa ICIDR program will promote the development of laboratory and clinical research capacity at KGH, with a particular emphasis on training Sierra Leonean research staff. The program will also enhance relevant scientific linkages between United States and KGH LF investigators, and encourage future collaborative relationships with other research groups leading to improvements in detection, prevention, amelioration, and treatment of Lassa fever in the subregion.

Lassa virus

Lassa virus (LASV), a member of the *Arenaviridae* family, is enveloped with a bisegmented, linear, single-stranded ribonucleic acid genome (Fig. 1). Virions are enveloped and pleomorphic, ranging in size from 80 to 150 nm. The virion envelope is a lipid bilayer unit membrane with approximately 10-nm external surface projections or spikes. Within the virion are 0–25 electron-dense "granules." The granules are usually described as host-derived ribosomes although rigorous characterization has not been performed. In thin-section electron microscopy the granules give a sandy-grained appearance to arenaviruses (Latin arenosus: sandy).

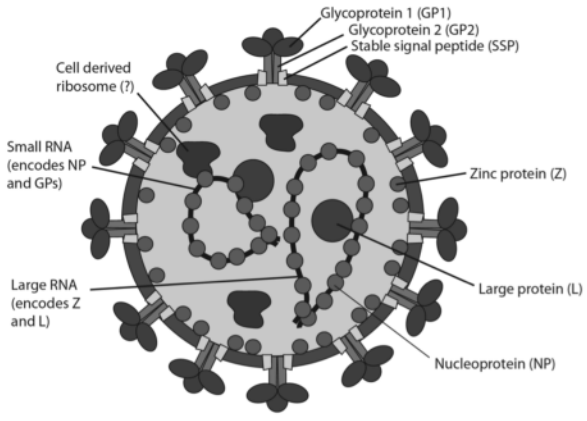

Figure 1. Lassa virus structure showing the organization of the surface glycoproteins GP1 and GP2, the nucleoprotein NP, the L and Z proteins. Also shown are the L and S RNA segments, which exist in circular complexes with large amounts of NP and small numbers of molecules of L protein.

Lassa virus contains structural proteins named: nucleoprotein NP (52 kDa), Zinc protein Z (11 kDa), and glycoproteins GP1 and GP2 (44 kDa and

38 kDa, respectively) (Fig. 1). The virion also contains an unusually long and stable signal peptide (SSP; 58 amino acids, 8 kDa). As detailed in the laboratory of Lassa ICIDR External Advisor Group (EAG) member Jack Nunberg (University of Montana), arenavirus SSP that is retained in the virion membrane forms a complex with GP2 [3]. A small number of copies of a large protein L (200 kDa), an RNA-dependent RNA polymerase, are also found in the virion. NP possesses group-specific antigenic determinants important for arenaviruses identification. GP antigens generally identify specific arenaviruses and determine neutralization.

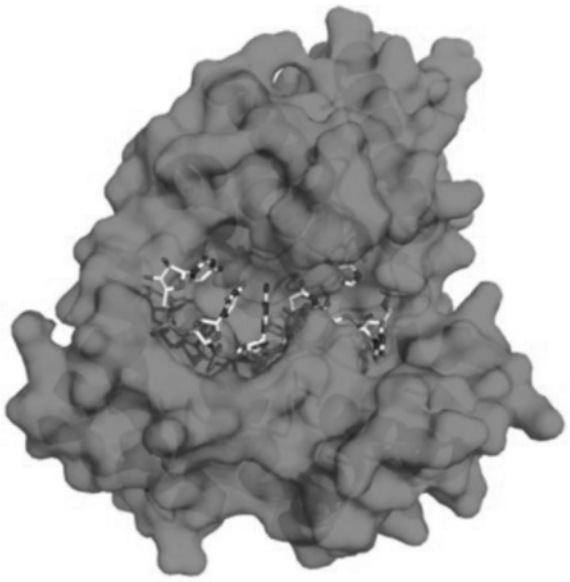

Figure 2. ssRNA (ball and stick) tunnels through a crevice in the N-terminal domain of LASV NP (green). NP structural studies by Lassa ICIDR EAG member Erica Ollmann Saphire and her post-doctoral fellow Kathryn Hastie (TRSI).

GP1 and GP2 are surface glycoproteins that are responsible for the spike-like projections on the virion observed in electron microscopy. In a major advance for the field, the X-ray crystallographic structure of the trimeric complex of arenavirus GP1/2 was recently solved in the laboratory of Lassa ICIDR EAG member Dr. Erica Ollmann Sapphire (The Scripps Research Institute, TRSI). GP2 contains a transmembrane domain that anchors it to the virion membrane. GP1 is associated with the viral membrane only by its noncovalent interaction with GP2. SSP has two membrane-spanning domains, and is retained in the GP complex through its interaction with the cytoplasmic tail of G2. Complementing these observations, Dr. Nunberg's group had previously demonstrated that Z and the GP2 cytoplasmic tail form a novel Zinc binding domain (ZBD), which binds two Zinc molecules [3].

NP is the most abundantly produced protein during viral infection (70% of virion mass). X-ray crystallographic studies in Dr. Saphire's laboratory by her post-doctoral fellow Kathryn Hastie demonstrated that NP is divided into N- and C-terminal domains [4-6]. The C-terminal domain functions as a 3'-5' exoribonuclease that specifically and rapidly digests double-stranded RNA (dsRNA) [4]. DsRNA is a key inducer of the interferon system and innate immunity. The exoribonuclease plays a key role in LASV-induced immunosuppression. Drs. Saphire and Hastie have also demonstrated that the N-terminal domain of NP binds RNA into a specific crevice [7] (Fig. 2). The mechanisms for oligomeric assembly of NP and the bound genomic RNA, within the ribonucleoprotein complex remain unknown.

The matrix protein Z has a length of less than 100 amino acids and occurs in monomeric and oligomeric forms. The glycine at position 2 (G2) is myristoylated, which is important for attaching to the plasma membrane (PM). The Really Interesting New Gene (RING) domain of Z contains a zinc-binding domain.

Replication

Cell entry by LASV depends on binding of GP1 to cell membrane receptors. The first cellular receptor discovered for LASV was α-dystroglycan (DG), a ubiquitous receptor for extracellular matrix (ECM) proteins [8]. Other cellular receptors for LASV potentially include the Tyro3/Axle/Mer (TAM) receptor tyrosine kinases Axl and Dtk (Tyro3), and the C-type lectins DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN) and LSECtin. LASV appears to enter smooth vesicles with transport to multivesicular bodies in the late endosome, thereby bypassing the early endocytic pathway [9]. GP2 is a class I viral fusion protein with a fusion loop and an otherwise predominantly helical ectodomain structure [10]. Exposure to acidic pH in the late endosome triggers conformation changes in GP2, six helix bundle formation, enabling fusion between the virion membrane and the endosomal membrane. This allows entry of the viral nucleic acids and proteins into the host cell cytoplasm.

LASV uses an 'ambisense' virus replication strategy common to all arenaviruses. The 5' end of the Small RNA is organized in the genomic sense and codes for the viral glycoprotein precursor (GPC). The 3' end is organized in the genome complementary sense (i.e. complementary to messenger RNA [mRNA]) and codes for NP. The two open reading frames are separated by a noncoding intergenic stem-loop. The loop appears to act as a transcription termination site during synthesis of mRNA rather than full-length RNA.

Similarly, the 3' end of the Large RNA is organized in a viral genome complementary sense and codes for L, while the 5 -end is organized in the genomic sense and codes for Z.

Once within the host cell cytoplasm, the viral RNA-dependent RNA polymerase L transcribes viral mRNAs and host ribosomes translate the viral mRNAs to produce NP as well as smaller quantities of L protein. Association of NP with nascent transcripts allows viral RNA replication ('secondary transcription') and production of full-length complementary RNA strands. Transcription of the complementary RNA of the Small RNA, results in mRNAs coding for GPC ('late transcription'). Transcription of the complementary RNA of the Large RNA results in mRNAs coding for Z. Translation of these mRNAs produces GPC and Z during 'late' transcription and translation. Z is thought to negatively regulate viral replication and transcription and is also important for the suppression of both viral and host cell translation. In addition to NP endonuclease activity defined by Saphire and Hastie discussed above, Z has also been implicated in antagonism of interferon-mediated antiviral responses [11].

Maturation of GPC involves proteolytic processing by the cellular signal peptidase and the proprotein convertase subtilisin kexin isozyme 1 (SKI-1)/site 1 protease (S1P), which yields a tripartite complex comprised of SSP, GP1 and GP2 in the early Golgi compartment. In contrast to other virus envelope glycoproteins, the arenavirus GP complex is unique in that it retains its cleaved signal peptide. The mature tripartite complex is ultimately trafficked to the plasma membrane, where virion assembly and budding occur [12]. SSP is crucial for maturation and appears to have a specific role in the stable prefusion conformation and transport of full-length GPC [13]. Lassa ICIDR EAG member Luis Branco (Zalgen) demonstrated the non-proteolytic secretory GP1 shedding occurs during expression of the arenaviral GP complex [14,15].

Because expression of Z alone can produce virus-like particles (VLPs), it is likely that Z drives the LASV virion budding process (Branco and others) [16,17]. Z has been demonstrated to interact with both NP and L, and myristoylated Z has been shown to interact with SSP of the GP complex. The budding process involves cellular proteins, such as Endosomal Sorting Complexes Required for Transport-III (ESCRT-III), and results in selective incorporation of certain cellular components, such as the aforementioned granules [18].

Epidemiology – Human infection

Lassa fever was first described in 1969 as a hospital-based outbreak in northeastern Nigeria. The previously undescribed febrile illness is named after a village called Lassa located in this region (Fig. 3). Persons living throughout West Africa are at continuous risk for Lassa fever. Because of its high lethality, ease of acquisition and potential for aerosol spread, Lassa virus (LASV) is a NIAID Category A pathogen and a highly credible bioterrorism threat.

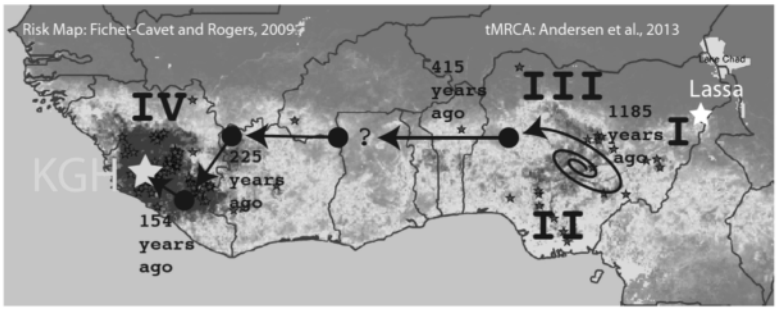

Figure 3. Risk map for Lassa fever showing the endemic area for Lassa fever [1]. Sites of the original case reports from near Lassa, Nigeria (white star), Kenema Government Hospital (KGH, yellow star) in Sierra Leone. KGH is situated in the region of the world with the highest number of LF cases (red areas of map). Distinct LASV lineages I-III circulate in Nigeria, while lineage IV is present in Sierra Leone and surrounding countries. Time to most recent common ancestor (tMCRC, L segment) analyses by Lassa ICIDR Advisory member Pardis Sabeti and her post-doctoral fellow Kristian Andersen indicate that LASV originated in Nigeria over 1100 years ago and spread to Sierra Leone about 150 years ago [2].

Transmission of LASV to humans occurs via direct contact with or ingestion of rodent excreta-contaminated materials. LASV is readily transmitted between humans via exposure to blood or body fluids, which makes nosocomial infection a matter of great concern. It has been estimated that there are up to 500,000 cases of Lassa fever per year in West Africa and 5,000 deaths [19-21]. These numbers are likely underestimates. Most LASV infected persons are never seen by health professionals due to poor access to medical facilities. Furthermore, the initial symptoms of Lassa fever are similar to other febrile illnesses and commonly misdiagnosed as malaria. The true incidence of Lassa fever has not been determined previously, in part

because of shortages in the supply of available diagnostic assays. Serosurveys have revealed high prevalences of LASV antibody in some populations where LF was infrequent or unknown, suggesting a high rate of LASV infection [22-24]. These studies support the concept that most infections with LASV result in asymptomatic or mild disease. The Lassa ICIDR at KGH will establish infrastructure, perform essential training and test the feasibility of performing accurate serosurveys of LASV exposure in Sierra Leone. For a further discussion of Lassa fever epidemiology in Sierra Leone please see Project 1.

Lassa fever – Natural history.

Infection with LASV can result in a range of outcomes from a mild or asymptomatic infection to a severe life-threatening illness. Signs and symptoms of Lassa fever occur 1-3 weeks after exposure and are highly variable. These can include fever, facial swelling, conjunctival injection, retrosternal, back or abdominal pain, sore throat, cough, vomiting, bleeding and diarrhea [25-27]. While temperatures as high as 40^{o}C have been reported, in many patients, including some with a self-reported perception of a high fever, the actual fever is mild (unpublished observations). Neurological problems have also been described following LASV infection including tremors, and encephalitis [28,29]. Temporary or permanent unilateral or bilateral deafness has been reported to occur in ~30% of patients with LF [28,29].

LASV and other arenaviruses infect and compromise endothelial cells, resulting in increased capillary permeability. The degree of specific organ damage is limited, even in fatal Lassa fever cases. This lack of gross pathological changes has also been observed in fatal disease induced by LASV in NHP [30]. Death from Lassa fever has been attributable to hypovolaemic shock, respiratory distress or severe encephalopathy, diminished effective circulating volume, shock, and multi-organ system failure [31]. Frank bleeding, usually mucosal (gums, etc.), does not occur in every case of severe disease, but confers a poor prognosis. Our studies [32,33] and those of others [21] suggest that the most accurate predictor of fatal outcomes in Lassa fever is high viremia. Patients with viremia levels greater than 5 X10^{3} infectious virions per ml were shown to have a poor prognosis. Other predictors of a poor prognosis are elevated liver enzymes and cytokine dysregulation [32,34]. Signs and symptoms in Lassa fever patients who survive typically begin to subside 2-3 weeks after onset, but full recovery can require many months or longer. We have observed high mortality rates in follow-up patients that survive the acute phase of the disease.

The case fatality rate (CFR) in Lassa fever patients presenting to health facilities has generally reported to be in the range of 15-20% [20,26,35,36]. During outbreaks the CFR has been reported to be approximately 50%. In more recent studies, the CFR in Nigerian Lassa fever patients diagnosed by polymerase chain reaction, which detects viremic subjects only, has been reported to be about 30% [37]. Our recently published study, which was lead by Jeffrey Shaffer, John Scheiffelin and Robert Garry (Tulane University) and Donald Grant and Humarr Khan (KGH) indicates that the CFR was approximately 70% in Sierra Leonean patients presenting to KGH in the post-conflict period with LASV viremia measured by antigen capture ELISA [33]. The comparison between the CFRs in these recent studies in Nigeria and Sierra Leone may reflect the different sensitivities of the assays used to measure viremia, differences in the LASV strains or lineages in the two countries or [as discussed below] differences in human genetics. These recent studies also should not be directly compared to prior studies, which showed CFR in the range of 15-20%. In most of these prior studies CFR were calculated based on both the presence of LASV viremia (which defines acute infection) and the presence of immunoglobulin M (IgM) antibodies, which in most viral infections signifies a recent infection. However, studies in both NHP and in

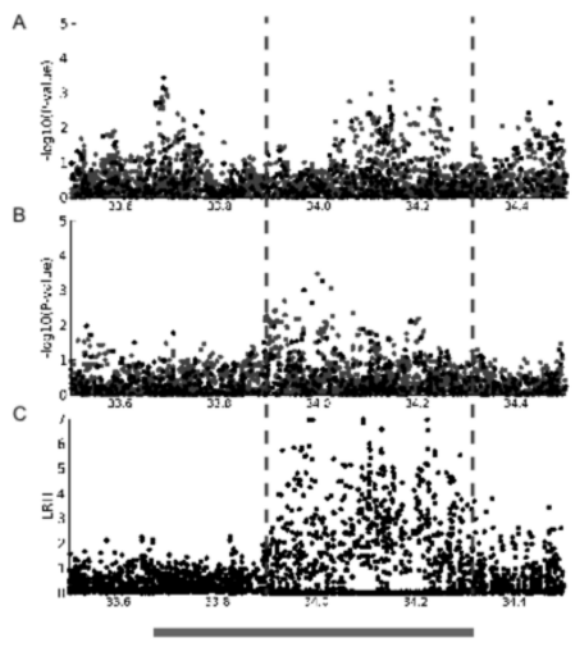

Figure 4. Overlap of the association signal with LF and the signal of positive selection in the Yoruba population of Nigeria at LARGE. **A)** Association with LF in a Sierra Leonean Mende cohort (~10% prevalence). **B)** Association with LF in the Nigerian Esan cohort (~21% prevalence). **C)** Signal of positive selection at LARGE in the Yoruba population of Nigeria (~30% prevalence). Blue bar indicates the position of the LARGE gene.

Lassa fever survivors suggest that immunoglobulin M antibodies is not be a reliable indicator of recent LASV infection, as this class of anti-LASV antibody persists for months or years after infection [32]. Thus, including antiLASV IgM+ patients as acute infections may underestimate the CFR. It should also be noted that CFR are based on patients presenting to health care facilities, which over-represented severe LASV infections, and do not include asymptomatic or mild infections that likely predominate in the wider population. Individuals that develop less severe manifestations of LASV infection or that remain asymptomatic do not usually seek medical care. Therefore, CFRs based patients presenting to health facilities do not reflect overall mortality rates caused by LASV infection, which are considerably lower.

The factors that determine whether a person develops a mild or severe disease after LASV infection are unknown. New insights by Lassa ICIDR EAG member Pardis Sabeti (Harvard University and the Broad Institute) suggest that the complexity in disease outcomes across LASV's endemic range are due in part to its influence on human evolution. The cellular receptor for LASV is the glycoprotein α-dystroglycan and its posttranslational modification by the protein LARGE is required for LASV entry [38,39]. During an exhaustive survey of human genetic variation in West African populations Dr. Sabeti and her group found evidence that the LARGE gene is under strong positive selection in the LF endemic zone [40]. Given the requirement for LARGE in LASV infectivity, it is plausible that the selection is driven by LASV resistance. Consistent with this, the frequency of the derived LARGE haplotype is highest in the Yoruban population of Nigeria (30%), but lower in the Esan of Nigeria (21%) and the Mende of Sierra Leone (10%), and absent in East Africans and non-Africans, populations where Lassa fever is absent [Sabeti et al., manuscript in preparation] (Fig. 4). This is congruent with results of LASV phylogenetic analyses performed by Dr. Sabeti's post-doctoral fellow Kristian Andersen, which indicate that LASV is oldest in Nigeria and appeared only "recently" circa 150 years ago in Sierra Leone (Fig. 3). Together these new observation might help to explain increased incidence of severe disease and higher CFRs in Sierra Leone compared to NIgeria.

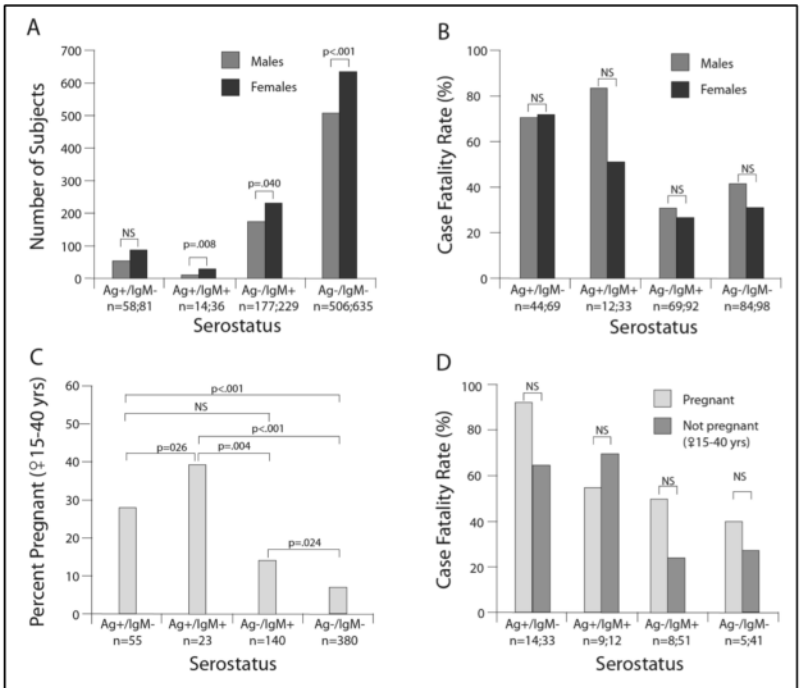

Figure 5**. Gender and self-reported pregnancy status of suspected Lassa fever cases presenting to the KGH Lassa Ward, 2008-2012.** Panel A: Frequency of suspected Lassa fever cases by gender and serostatus. Panel B: Cases fatality rates by gender and serostatus. Panel C: Percentage of female patients of childbearing age with self-reported pregnancy status by serostatus. Panel D: Case fatality rates in female patients with self-reported pregnancy status.

The antiviral drug ribavirin, in its intravenous form is effective in the treatment of LF, particularly when given early in the course of illness [21,41]. However, the drug is expensive and is not yet licensed by the United States Food and Drug Administration (FDA) for general use. Maintenance of appropriate fluid and electrolyte balance, oxygenation and blood pressure may also improve survival in Lassa fever. For additional discussion of ribavirin efficacy and the need to establish clinical trials capacity at KGH please see Project 2.

Lassa fever in pregnant women and in children

LASV infection in persons with altered or naïve immune status, such as in pregnancy or childhood, appears to lead to more severe disease. In a recent study lead by Lassa ICIDR Co-PI Jeff Shaffer we found that women who self-reported as pregnant were significantly overrepresented among women that present with active LASV infection to KGH in the post-conflict period [33] (Fig. 5). This study is consistent with prior observations indicating that Lassa virus infection in pregnancy is usually associated with grave outcomes [42,43]. In essentially 100% of cases of LASV infection in the third trimester fetal death occurs. It has been reported that in the eastern province of Sierra Leone, Lassa virus infection may account for a high percentage (70%) of spontaneous abortions that occur [42,44]. The cause of death of the fetus has not been determined, but could result from direct effects of the LASV on the developing fetus or the placenta that thereby compromises the supply of food, nutrients and oxygen. LASV

can be detected in cord blood, placenta and in aborted fetuses. Pregnant women infected with LASV also have high rates of mortality, which has been reported to be as high as 90% in the third trimester of pregnancy [42,43]. It should also be noted that high viremia levels in the placenta and the products of conception put healthcare providers in maternity clinics at considerable risk for infection. Lassa fever is also a disease of importance for children, who represent a disproportionate number of fatal Lassa fever cases presenting to KGH in the post-conflict period [33].

Epidemiology-Rodent infection

The natural reservoir of LASV is *Mastomys natalensis*, the natal mastomys or multimammate "rat" [45] (Fig. 6). *Mastomys natalensis* often live or forage in human homes, facilitating LASV transmission to humans [46,47]. They are nocturnal, hide in dark spaces during the day, and are noted for their fecundity. Females have 12 pairs of mammary glands and an average litter of 6–12. Historically, there has been uncertainty about whether or not LASV is restricted to a single species of the genus *Mastomys* throughout West Africa, but molecular genotyping has resolved this issue [48]. LASV has also been detected in *Rattus* and *Mus*, albeit rarely, raising the possibility that other rodents could be involved as transient or intermediate vectors.

Figure 6. *Mastomys natalensis* (natal mastomys aka multimammate "rat") is the main reservoir of Lassa virus. Mastomys are peridomestic and highly prolific breeders. Food stored in case homes often shows evidence of rodent entry. Lassa fever is acquired through contact with excreta or preparation of "rat" for food. Photo credit Lina Moses Service and Training Core Co-PD.

Mastomys natalensis show no overt symptoms of LASV infection, but shed the virus in saliva, urine and feces. It has been suggested that *Mastomys* are congenitally or perinatally infected with LASV, and remain persistently infected through their lifespan (approximately 1 year in the wild). Only limited laboratory studies of LASV infection in Mastomys have been conducted to address this possibility; Infection *in utero* or at birth did not lead to overt pathology and rodents remained viraemic and viruric for their life times. While this characteristic has been observed in other arenaviruses, such as when lymphocytic choriomeningitis virus (LCMV) in fects its natural; host *Mus musculus* (house mouse), more studies are needed to determine if LASV persistence also occurs in free-living *M. natalensis*. It is possible that maintenance of LASV in *M. natalensis* populations involves infection and transmission of the virus amongst animals of various ages, and that infections in older animals are transient. As discussed, transmission of LASV from *M. natalensis* to humans occurs via direct contact with rodent urine, feces, and saliva, or by contact or ingestion of excretion-contaminated materials. Infection may also occur when *M. natalensis* are caught and prepared as food.

Mastomys natalensis is among the most prevalent rodents in subSaharan African. They are common throughout grassland and tropical secondary forest areas. There is not an obvious correlation with the distribution of Lassa fever cases, which is extremely heterogeneous even within apparently identical ecosystems and reservoir distributions. [45-47,49]. Nevertheless, it seems reasonable that variation in the prevalence of *M. natalensis* and/or LASV infection in *M. natalensis* populations might explain some of the patchy distribution observed for Lassa fever across West Africa [24,50].

Significant temporal variation in the presentation of Lassa fever patients has been noted. While cases present year-round in both Sierra Leone and Nigeria, which record the highest number of cases, peak incidence usually in both countries usually occurs during the dry season. The traditional harvest season also corresponds to this period, suggesting that improper storage of the grains may result in an increase in rodent populations in contact with humans at this time [49,51]. *Mastomys natalensis* also breed primarily at the end of the rainy season, potentially introducing a large number of LASV-excreting juveniles into the population in the dry season.

Laboratory Diagnosis

The non-specific clinical presentation of Lassa fever makes this disease extremely difficult to recognize on clinical grounds alone, especially in the early phases. Prompt laboratory diagnosis is therefore essential. Laboratory diagnosis of a viral infection relies on the demonstration of virus antigens or nucleic acids and/or virus-specific antibodies. Infection can be also confirmed by growing the virus in cell culture, however, in clinical settings there is urgency in diagnosing the disease and initiating aggressive treatment that cannot be delayed by transport of samples to containment laboratories and infection of permissive cell cultures for evaluation of the infectious agent. Diagnostic assays for LF have been based on LASV grown in BSL-4 laboratories. The processes for producing the traditional enzyme-linked immunosorbent assay (ELISA)-based assays are expensive in terms of time and human resources, and consequently have always been in short supply in West Africa. Recently, through an effort of a the Tulane team, KGH investigators and other members of the VHFC (vhfc.org), LASV antigen (Ag)-capture and IgM- and IgG-capture ELISA using recombinant LASV proteins have been produced that have similar or better sensitivity, specificity and efficiency than the BSL-4 assays [52]. A lateral flow immunoassay (LFI) for LASV antigen has recently been developed that can reliably detect LASV antigen at the bedside from a drop of blood obtained by a simple fingerstick. We propose that the LFI can be disseminated to outlying clinics and laboratories and reliably and safely performed by persons with limited laboratory training. Feasibilty of this proposition, which could have profound implications for Lassa fever control, will be developed in studies planned under Project 1.

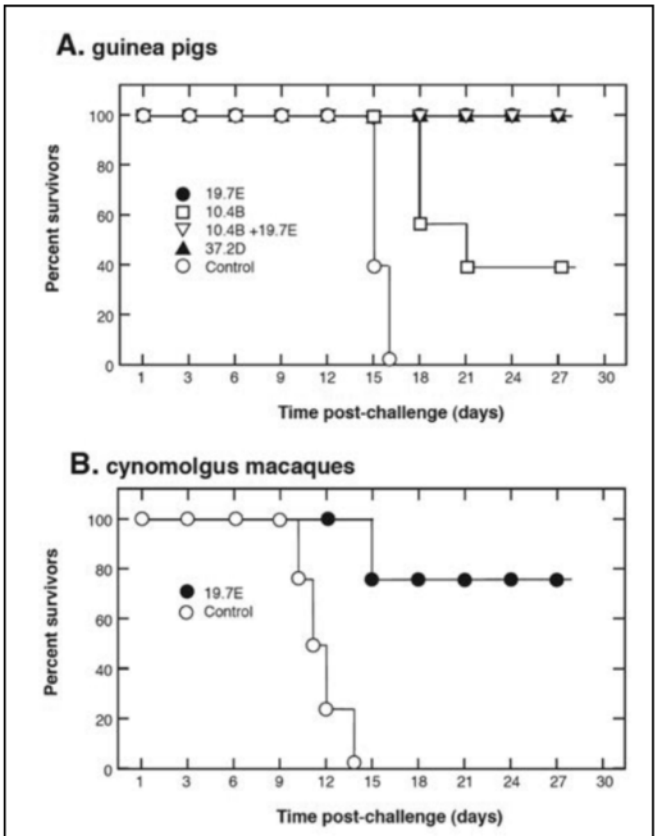

Figure 7. Protection from lethal infection in two animal models of fatal Lassa fever. Panel A. Survival curves for GPs treated with 30 mg/Kg and 15 mg/Kg of 19.7E and 10.4B, or 30 mg/Kg of 19.7E, 10.4B, and 37.2D individually, and challenged with a LASV mutant that is uniformly lethal for outbred guinea pigs. Panel B. Cynomolgus macaques were challenged with a lethal dose of Lassa virus. 30 mg/Kg of 19.7E was administered on days 0 and 5 post-infection.

Research to produce Lassa fever vaccines and new therapeutics

Repeat LASV infections in survivors appear to be very rare, if they occur at all [33]. This suggests that an effective Lassa fever vaccine may be feasible. Further support for the possibility of a Lassa fever vaccine comes from experience with Junin virus (JUNV), a New World arenavirus that is the causative agent of Argentine hemorrhagic fever. Candid 1, a live attenuated JUNV vaccine [53], proved safe and highly efficacious in the AHF-endemic region. A vaccine for Lassa fever would be highly valuable for Sierra eonean and other West African populations and for persons traveling to West Africa. Lassa fever vaccine development efforts will benefit by a better understanding of the immune responses required to protect from LASV infection and disease. In general, protective immunity has been more closely correlated with cellular immune responses to LASV rather than humoral responses. It should be noted that to date human studies of the roles of innate, cellular and humoral immunity in Lassa fever survival have been limited in scope.

Several approaches to develop a Lassa fever vaccine have been tested including replication-competent viral vector approaches, such as one based on based on vesicular stomatitis virus (VSV) [54]. A LASV-Mopeia hybrid virus has also been shown to protect against LASV infection in animal models [55]. Lassa ICIDR EAG member Luis Branco (Zalgen) has developed a replication-deficient virus-like particle (VLP) LASV vaccine platform [17]. Lassa ICIDR EAG member Katherine Cashman (United States Army Medical Research Institue for Infectious Diseases, USAMRIID) has developed a DNA vaccine expressing LASV GPC, which was found to offer complete protective immunity to guinea pigs and *Cynomolgus macaques* when delivered by intradermal electroporation [56].

Infected humans and experimental animals such as nonhuman primates (NHP) develop strong antibody responses to LASV, but those antibodies are not associated with viral clearance. Low levels of LASV neutralizing antibodies, if detected at all, are usually only present after recovery. Nevertheless, antibodies may contribute to Lassa fever survival, as antibodies can mediate passive protection in other arenavirus infections such as AHF [57], and in experimental Lassa fever. Passive transfer of antibodies is used as a treatment for AHF [58]. Passive antibody transfer has also been shown to have efficacy in animal models of LF [59] and in limited human studies [60]. Although anecdotal, passive antibody is credited with saving the life of Jordi Casals-Ariet, the Yale virologist who discovered LASV [61], but contracted LF during his initial characterization of the virus (a Yale lab technician died earlier from LF). The plasma transfused was donated by Lily Pinneo, a nurse who had contracted LF in the 1969 Nigerian outbreak and survived. Human immune plasma containing neutralizing antibodies against JUNV is used for post-exposure protection against AHF [20].

Despite the fact that LASV neutralizing antibodies are a small subset of the antibodies produced (~10%) during natural LASV infection, we have been successful in deriving twelve independent neutralizing MAbs against lineage IV LASV strains to date. To facilitate studies of the protective roles of antibodies in LF, Branco developed novel methods to express huMAbs (gram quantities can be obtained from a shaker flask). Our capacity to rapidly produce huMAbs to scale permits studies of huMAbs as passive antibodies in LF animal models. We observed 100% protection from lethal challenge with LASV in a newly developed outbred guinea pig model after a single injection of neutralizing huMAbs 19.7E (an anti-GP1 antibody) or 37.2D (an anti-GP2 antibody). A third human antibody, 10.4B conferred partial protection (2/5 animals, 40%) from lethal LF in guinea pigs. Significant protection was also observed in a nonhuman primate model of LF. Three out of four (75%) cynomolgus macaques treated with 19.7E survived lethal challenge with LASV, without evidence of any signs or symptoms. Time to death in the fourth animal was delayed relative to control NHPs. More potent huMAbs than 19.7E, 10.4B or 37.2D have now been derived, and other antibodies perhaps recognizing different B cell epitopes, alone or in combination may mediate even better protection.

Small-molecule compounds that specifically inhibit arenavirus entry have recently been described, and show promise for treatment and post-exposure prophylaxis. For example, ST-193, has a 50% inhibitory concentration (IC50) of 1.6 nM against LASV pseudotypes. ST-193 also inhibited pseudotypes generated with other arenavirus envelopes as well, those of Junín, Machupo, Guanarito, and Sabiá viruses [62]. ST-193 appears to stabilize the prefusion GPC complex against acidic pH. Other small molecules that block LASV entry include include ST-294, 8C1 and 17C8 [63]. ICIDR EAG member Nunberg [64] has been involved in development of a potential anti LASV drug named "T-705 (Favipiravir) that targets the viral RNA-dependent RNA polymerase [63].

Lassa ICIDR overall hypothesis: Further development of research capacity, with emphasis on training Sierra Leonean staff, will permit the KGH Lassa fever research program to emerge as an exceptional resource for human clinical trials of NIAID's promising portfolio of Lassa fever diagnostics, therapeutics, immunotherapeutics and vaccines.

Existing infrastructure and capabilities.
The infrastructure and resources of our public health and research network in Sierra Leone developed over the past decade offer access to human subjects in the LASV endemic zones of West Africa, and one of the only opportunities in the world to conduct detailed analysis of the epidemiology and natural history of a severe VHF [65,66]. KGH is a 350-bed facility that currently maintains a 25-bed Lassa Ward and full-time staff completely dedicated to the care of patients with Lassa fever. Working closely with the Sierra

Figure 8. Current progress on construction of new Lassa Ward. This is the view from the rear of the High Containment wing, with the Administration Building on the right. Construction is projected to be complete by August 2014 (photo credit RFG; 28 Feb 2014).

Leone Ministry of Health and Sanitation [MOHS], we are nearing the final stages of construction of a new 48-bed Lassa Ward that will replace this timeworn facility. The completion of construction of this new facility is timely and will greatly faciltate achieving the goals of the Lassa ICIDR (Fig. 8).

Also in place is a highly functional system for active and passive surveillance of Lassa fever cases. This surveillance work is lead by Lansana Kanneh is conducted by a team of outreach workers working in collaboration with local health centers. Lassa fever is a zoonotic infection, transmitted to humans by *Mastomys natalensis* (multimammate or bush rat), and there is an active ecology team at KGH that has decades of experience in trapping rodents and other mammals. Collectively the ecology group has over 75 years of combined experience.

Fig. 9. The Lassa Laboratory at Kenema Government Hospital.

The Research Laboratory, also situated on the KGH grounds, was completed in 2005 (Fig. 9). The Laboratory performs flow cytometry, cytokine analyses, ELISpot, real-time and conventional PCR, clinical chemistries, hematology, and a variety of immunoassays. The KGH Laboratory is equipped with liquid nitrogen (LN2) tanks, LN2 dry shippers, centrifuges, a solar battery bank, an advanced water purification system, high-speed Internet connectivity, computers, electronic databases and apparatus for storing, processing, managing and shipping biospecimens. The Laboratory is lead by Mr. Augustine Goba and is staffed with highly experienced technicians, who have worked closely with Tulane scientist to develop the current generation of Lassa feve diagnostic, employed for patient evaluation. The KGH will now further development as epidemiological and clinical management tools in studies proposed in Projects 1 and 2.

Overall structure of the Lassa ICIDR

The research team from KGH has over 10 years of experience partnering with Tulane University and other partners to develop and maintain its laboratory and clinical infrastructure. We are ready to make a major step forward to confront the challenges of Lassa fever, namely developing the capacity to perform human clinical trials. The International Collaboration in Infectious Disease Research on Lassa fever (Lassa ICIDR) is composed of an Administrative Core and two projects. Project 1 will evaluation next-generation Lassa fever recombinant antigen immunodiagnostics, including point-of-care diagnostics (Fig. 10). Project 2 will expand clinical research capacity at KGH (Fig. 11). The Administrative Core will facilitate communication, planning, data sharing, and scientific and fiscal oversight of the component research projects of the Program.

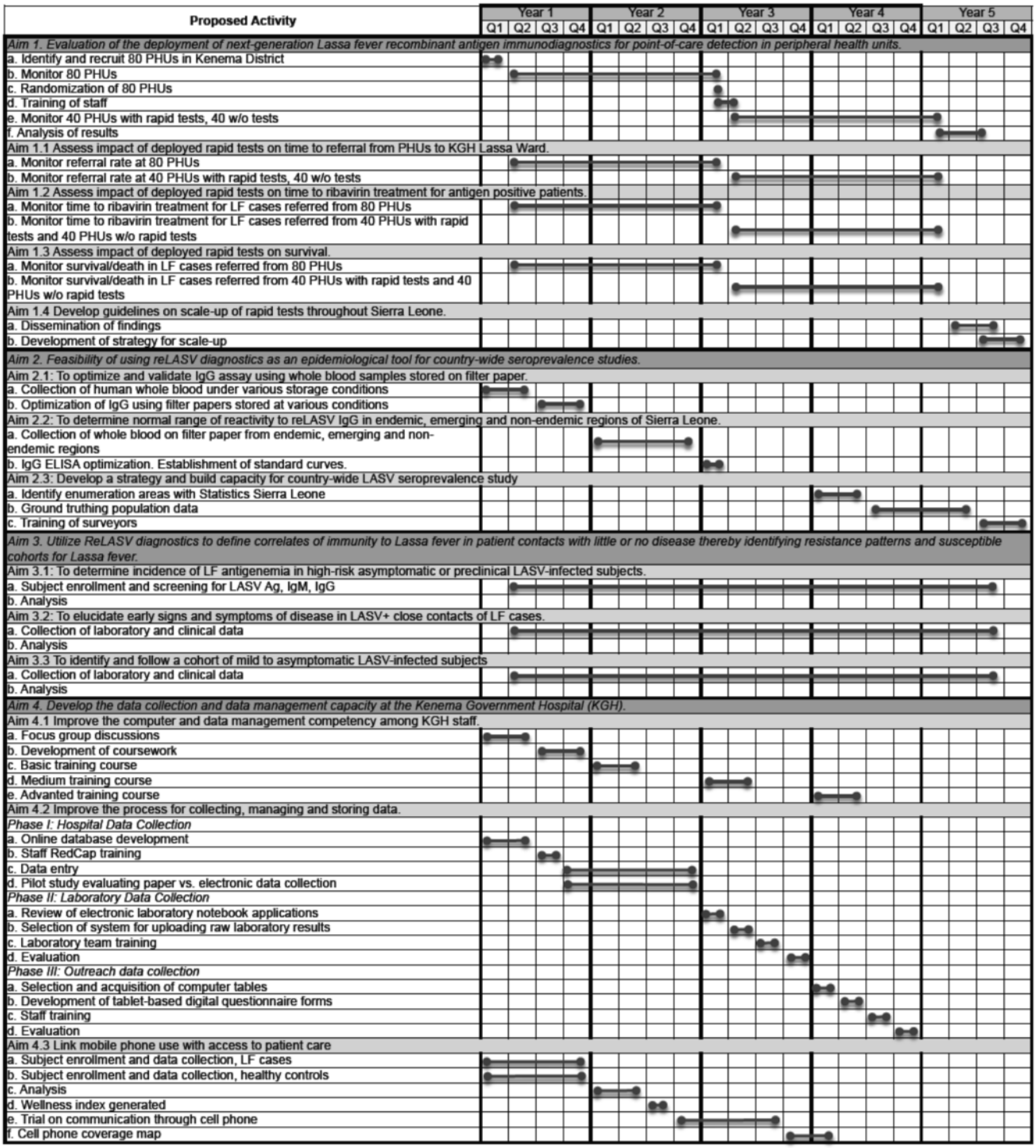

Fig. 10. Timeline for Project 1.

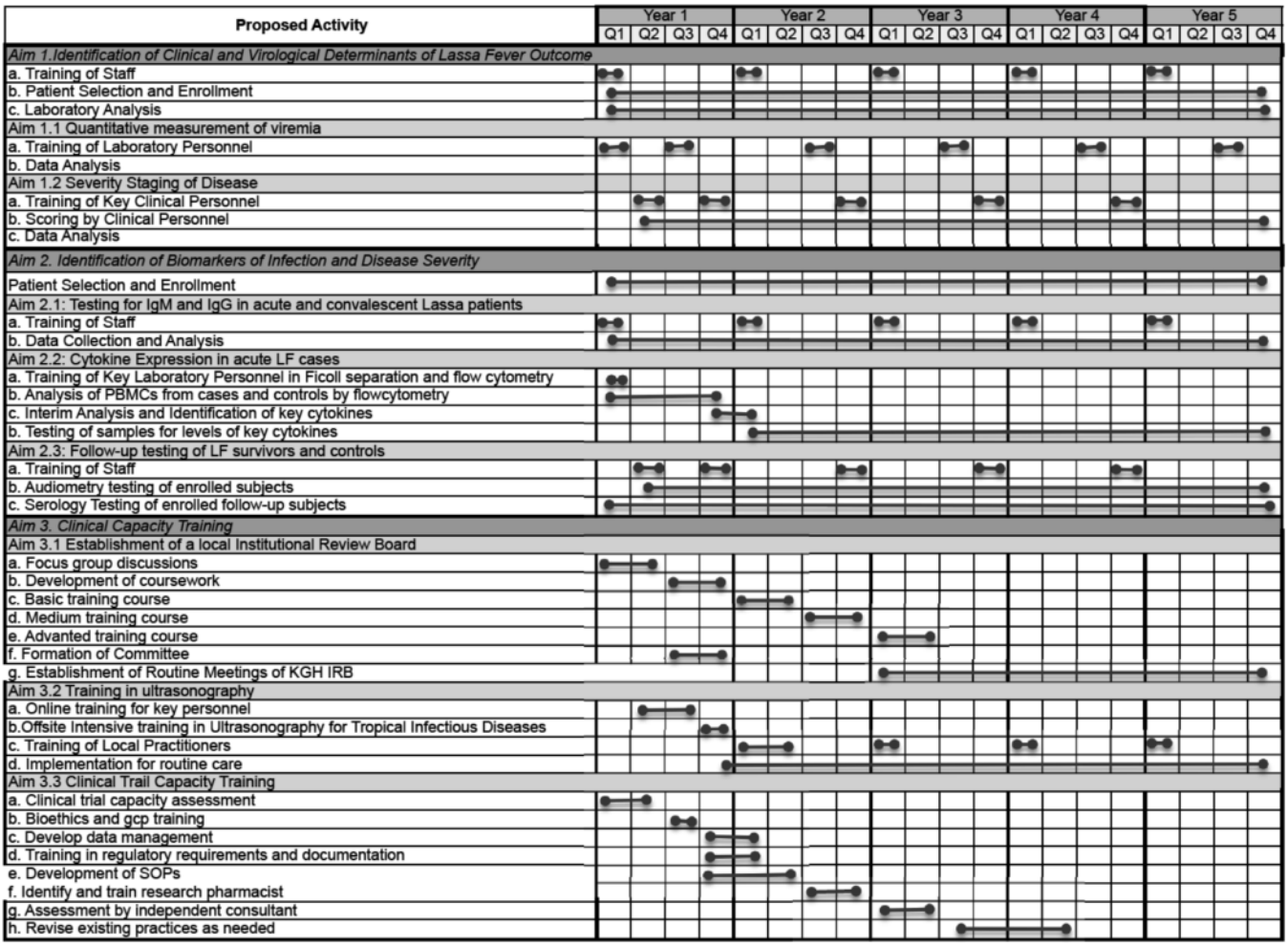

Fig. 11. Timeline for Project 2.

This is the first submission by Tulane University and the Lassa Fever Program at Kenema Government Hospital to the International Collaborations in Infectious Diseases Research (U19) program.

A Progress Report Publication List is not applicable.

Human subjects research conducted under Project 1 and 2 meets the definition of “Clinical Research.” Therefore, the research protocols and all consent/assent and data forms will be submitted to and approved by the Institutional Review Board (IRB) of Tulane and the Ethics Committee of Sierra Leone prior to implementation of the study (see Table below). Informed consent will be obtained from all subjects prior to obtaining specimens for research. In some cases, execution of the study may entail testing anonymous sample remainders or serum specimens collected for other purposes. When appropriate, exemption from the requirement of IRB/Ethics Committee approval will be sought and obtained prior to testing the samples.

Collaborating sites and assurance numbers for Human Subjects research		
Site	**IRB Location**	**Federalwide assurance number**
Tulane University	New Orleans, LA	FWA00002055
Kenema Government Hospital	Freetown, Sierra Leone	FWA00019671

The Administrative Core will provide the investigators of Projects 1 and 2 regulatory and ethical compliance support for this aspect of their research. Tulane University will provide oversight and Institutional Review Board review of all human subjects research conducted for the Lassa ICIDR. Tulane University policy requires that all research in which it participates must be reviewed by its own IRB. Research involving human participants is an important part of the overall research program at Tulane University. Tulane University and its employees are required to comply with certain policies and procedures as published in the Code of Federal Regulations by the Department of Health and Human Services (26 January 1981) (45 CFR Part 46) and Food and Drug Administration (27 July 1981) (21 CFR Parts 50 & 56) regarding the rights and welfare of human subjects at risk, involved in activities supported by grants and contracts from the Department of Health and Human Services, the Food and Drug Administration, and certain other agencies providing funds to support investigations involving human subjects. The Tulane University IRB shall determine whether these subjects will be placed at risk and, if risk is involved, whether: the risks to the subject are so outweighed by the sum of the benefit to the subject and the importance of the knowledge to be gained as to warrant a decision to allow the subject to accept these risks; the rights and welfare of any such subjects will be adequately protected; legally effective informed consent will be obtained by adequate and appropriate methods in accordance with provisions of applicable law, and the conduct of the activity will be reviewed at timely intervals.

Tulane University acknowledges and accepts its responsibilities for protecting the rights and welfare of human subjects of research covered by this assurance. It is the policy of Tulane University that all research involving human subjects will be reviewed by an IRB that has been established under an assurance of compliance negotiated with the Department of Health and Human Services (DHHS). Except for those categories specifically exempted by this assurance, the involvement of human subjects in research will not be permitted until the IRB has reviewed and approved the research protocol (study design) and consent form, and informed consent has been obtained in accordance with and to the extent required by federal regulations. The IRB reviews ongoing research at appropriate intervals; but in no event, less than once per year. The interval for continuing review will be determined by the IRB. This assurance is applicable to all activities which, in whole or in part, involve research with human subjects if: the research is sponsored by this institution; the research is conducted by or under the direction of any employee or agent of this institution in connection with his or her institutional responsibilities; the research is conducted by or under the direction of any employee or agent of this institution using any property or facility of this institution; or the research involves the use of this institution's non-public information to identify or contact human research subjects or prospective subjects.

Please refer to the Human Subjects section of Projects 1 and 2 for information about risks to the subjects, adequacy of protection against risks, potential benefits of the proposed research to the subjects, and Importance of the knowledge to be gained.

Human Subjects research conducted in Projects 1 and 2 will involve approximately equal numbers of male and female subjects. Since the subject enrollment will take place in West Africa, 100% of the study population is expected to be black African. There are no exclusions. Please refer to the Human Subjects sections of Projects 1 and 2 for further information about inclusion of Women and Minorities.

Children of all ages will be included in the Human Subjects research conducted in Projects 1 and 2. The research team includes healthcare workers and facilities with extensive experience in dealing with children. Please refer to the Human Subjects sections of Projects 1 and 2 for further information about inclusion of children.

Project 1 involves the use of Vertebrate Animals in Research. Please refer to the Vertebrate Animal section of Project 1 for the 5 points. Please also note that the Administrative Core will ensure that all Lassa ICIDR animal research is conducted according to applicable laws and regulations. The Administrative Core will also provide training sessions in the ethical use of animals in research.

Please refer to the Administrative Core for details regarding Select Agent Research.

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The Administrative Core will manage the Consortium Agreement between Tulane University and the Lassa Fever Program at Kenema Government Hospital.

Please refer to the Administrative Core for details regarding the Consortium Agreement.

All RFA required Letters of Support from Institutional and Government officials are compiled in the Administrative Core section of this application.

Please refer to the Administrative Core for details regarding Resource Sharing.

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Tulane University
Duns Number:	0537858120000
Street1*:	1430 Tulane Avenue
Street2:	
City*:	New Orleans
County:	Orleans
State*:	LA: Louisiana
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	701122699
Project/Performance Site Congressional District*:	LA-002

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Kenema Government Hospital
DUNS Number:	8505106210000
Street1*:	1 Combema Road
Street2:	
City*:	Kenema
County:	
State*:	
Province:	
Country*:	SLE: SIERRA LEONE
Zip / Postal Code*:	
Project/Performance Site Congressional District*:	00-000

File Name

Additional Location(s)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ○ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ● No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ● **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number 00002055

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number A4499-01

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ● **Yes** ○ **No**

6.a. If yes, identify countries: Sierra Leone

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	INTRO_ICIDR_Abstract.pdf
8. Project Narrative*	Overall_Narrative_1.pdf
9. Bibliography & References Cited	INTRO_ICIDR_references.pdf
10.Facilities & Other Resources	
11.Equipment	

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

International Collaboration in Infectious Disease Research on Lassa fever (Lassa ICIDR)

Lassa fever is an acute and often-fatal hemorrhagic disease caused by Lassa virus (LASV), an arenavirus. Lassa fever is a zoonotic infection, transmitted to humans by *Mastomys natalensis* (the multimammate “rat”). The highest incidence of Lassa fever in the world is in the Eastern Province of Sierra Leone. Signs and symptoms of LF, which occur 1-3 weeks after virus exposure, are highly variable, and can include fever, facial swelling, conjunctival injection, and vomiting. Frank bleeding occurs in less than a third of cases, but confers a poor prognosis. Signs and symptoms in LF patients who survive begin to subside 2-3 weeks after onset, but full recovery can require many months or longer. Kenema Government Hospital (KGH) was an important site for Lassa fever clinical and laboratory research throughout the 1970s and 1980s. The violent civil conflict in Sierra Leone from 1991 to 2002, sometimes referred to as the Blood Diamonds War, forced suspension of Lassa fever research at KGH in 1993. Following cessation of hostilities, our consortium of Lassa fever researchers and other partners began rebuilding the scientific infrastructure at KGH, with a major focus of the development of improved laboratory diagnosis for Lassa fever. There is no vaccine for LF and the efficacy of the antiviral drug ribavirin in treating LF remains a matter of controversy. There is an urgent need to develop epidemiological and clinical measures to combat the public health challenges poised by Lassa fever in Sierra Leone and across West Africa. Promising next-generation Lassa fever diagnostic immunoassays, including rapid tests, require further development as epidemiological and clinical management tools.

The overall hypothesis of the Lassa ICIDR is that further development of research capacity, with emphasis on training Sierra Leonean staff, will permit the KGH Lassa fever research program to emerge as an exceptional resource for human clinical trials of NIAID’s promising portfolio of Lassa fever diagnostics, therapeutics, immunotherapeutics and vaccines. The Overall Specific Aims (listed in priority order) are: Aim 1. Further enhance and utilize the symmetrical and highly productive partnership developed over the last decade between Tulane University and the Lassa fever program at KGH; Aim 2. Promote the development of laboratory and clinical research capacity at KGH, with a particular emphasis on training Sierra Leonean staff; and Aim 3. Encourage future collaborative relationships with other research groups leading to improvements in detection, prevention, amelioration, and treatment of Lassa fever in the subregion.

Research question(s) to be addressed in the Lassa ICIDR Projects include for Project 1: Can second generation Lassa fever recombinant immunoassays be used effectively as point-of-care diagnostics and surveillance tools for Lassa fever, and for Project 2: What are the clinical and virological determinants of Lassa Fever outcome and is it possible to identify biomarkers of LASV infection and Lassa Fever outcome. Through the proposed research we will acquire new information regarding the natural history of Lassa fever and the demographic distribution of people exposed to LASV using second generation Lassa fever recombinant immunoassays. We will also elucidate risk factors for acquiring serious or fatal LASV infection. This new information will guide evidence-based investments for public health programming and policy. We will also define biomarkers of Lassa fever pathobiology through the course of illness. Identification of these factors could lead to evidence-based approaches to reduce mortality from Lassa fever. We also propose to take a major step forward to confront the challenges of Lassa fever, namely developing the capacity to perform human clinical trials.

International Collaboration in Infectious Disease Research on Lassa fever (Lassa ICIDR)

Through the proposed research we will acquire new information regarding the natural history of Lassa fever and the demographic distribution of people exposed to LASV using second generation Lassa fever recombinant immunoassays. We will also elucidate risk factors for acquiring serious or fatal LASV infection. This new information will guide evidence-based investments for public health programming and policy. We will also define biomarkers of Lassa fever pathobiology through the course of illness. Identification of these factors could lead to evidence-based approaches to reduce mortality from Lassa fever. We also propose to take a major step forward to confront the challenges of Lassa fever, namely developing the capacity to perform human clinical trials.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Robert Middle Name F Last Name*: Garry Suffix:

Position/Title*:
Organization Name*: TULANE UNIVERSITY
Department:
Division:
Street1*: TULANE UNIVERSITY
Street2: DEPARTMENT OF MICROBIOL/IMMUNOLOGY
City*: NEW ORLEANS
County:
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 701122613

Phone Number*: (504) 988-2027 Fax Number: (504) 988-1994 E-Mail*: rfgarry@tulane.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: PD/PI Other Project Role Category:

Degree Type: Degree Year:

	File Name
Attach Biographical Sketch*:	Garry_biosketch_for_ICIDR.pdf
Attach Current & Pending Support:	

Tracking Number: GRANT11602615
Funding Opportunity Number: RFA-AI-14-002 . Received Date: 2014-03-07T14:26:44.000-05:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Sheik Middle Name Humarr Last Name*: Khan Suffix: M.D.

Position/Title*: Director
Organization Name*: Kenema Givernment Hspital
Department: Lassa Fever Program
Division:
Street1*: 1 Combema Road
Street2:
City*: Kenema
County:
State*:
Province:
Country*: SLE: SIERRA LEONE
Zip / Postal Code*:

Phone Number*: 232 78 627 621 Fax Number: E-Mail*: PII

Credential, e.g., agency login:

Project Role*: Co-PD/PI Other Project Role Category:

Degree Type: MBChB Degree Year:

File Name

Attach Biographical Sketch*: biosketch_of_Humarr_Khan_final.pdf

Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: First Name*: John Middle Name Scribner Last Name*: Schieffelin Suffix:

Position/Title*: Assistant Professor of Pediatrics
Organization Name*: Tulane University
Department:
Division:
Street1*: 1430 Tulane Ave
Street2: TB-8
City*: New Orleans
County:
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 701120000

Phone Number*: 5049885117 Fax Number: 5049882613 E-Mail*: jschieff@tulane.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Co-PD/PI Other Project Role Category:

Degree Type: MD,AB,MSPH Degree Year: 2001, 1992, 1995

File Name

Attach Biographical Sketch*: Schieffelin_Biosketch_022714.pdf

Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Jeffrey Middle Name George Last Name*: Shaffer Suffix:

Position/Title*:	Assistant Professor
Organization Name*:	Tulane University
Department:	Biostatistics
Division:	Public Health/Trop. Medicine
Street1*:	1440 Canal Street
Street2:	
City*:	New Orleans
County:	Orleans
State*:	LA: Louisiana
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	701122613

Phone Number*: 5044328658 Fax Number: E-Mail*: jshaffer@tulane.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Co-PD/PI Other Project Role Category:

Degree Type: BS, MS, PhD Degree Year: 1999, 2001, 2007

	File Name
Attach Biographical Sketch*:	Jeff_Biosketch.pdf
Attach Current & Pending Support:	

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: Robert
Middle Name: F
Last Name*: Garry
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ○ No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ● No

If the answer is "Yes" then please answer the following:

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0537858120000

Legal Name*: TULANE UNIVERSITY
Department:
Division:
Street1*: TULANE UNIVERSITY
Street2: 6823 ST. CHARLES AVE
City*: NEW ORLEANS
County:
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 701180000

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
Ms.	Kathleen	M	Kozar	

Position/Title: Director
Street1*: 1430 Tulane Avenue, Ep-15
Street2:
City*: New Orleans
County: Orleans
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 701122613
Phone Number*: 5049885613 Fax Number: 5049881748 Email: elecnot@tulane.edu

7. TYPE OF APPLICANT*

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Lassa ICIDR - Administrative Core

12. PROPOSED PROJECT

Start Date*	Ending Date*
01/01/2015	12/31/2019

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	TULANE UNIVERSITY
Duns Number:	0537858120000
Street1*:	TULANE UNIVERSITY
Street2:	6823 ST. CHARLES AVE
City*:	NEW ORLEANS
County:	
State*:	LA: Louisiana
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	701180000
Project/Performance Site Congressional District*:	LA-002

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Kenema Government Hospital
DUNS Number:	8505106210000
Street1*:	1 Combema Road
Street2:	
City*:	Kenema
County:	
State*:	
Province:	
Country*:	SLE: SIERRA LEONE
Zip / Postal Code*:	
Project/Performance Site Congressional District*:	

File Name

Additional Location(s)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ● **Yes** ❍ **No**

6.a. If yes, identify countries: Sierra Leone

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	ADMIN_CORE_ICIDR_Abstract.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Admin_core_references.pdf
10.Facilities & Other Resources	
11.Equipment	

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

International Collaboration in Infectious Disease Research on Lassa fever (Lassa ICIDR)

An efficient administrative structure is essential to the design and implementation of this U19 Program. The Lassa ICIDR Administrative Core is designed to facilitate communication, planning, data sharing, and scientific and fiscal oversight of the component research projects of the Lassa ICIDR. Through the Administrative Core will oversee all the goals of Lassa ICIDR Program. The Core will fund the annual travel of the External Advisory Board to the Program site of review. The Core Program Managers will coordinate, schedule, and implement minimum monthly internal meetings and teleconferencing among the Program sites, and will assist the Co-PDs of the Projects in the preparation of progress reports, internal compliance filings and edit manuscripts to support the Program. The Core will oversee data sharing, data archiving, and overall record keeping of the Program. The Administrative Core will be responsible for the overall organization, management, decision-making, and evaluation of the Lassa ICIDR with the following Specific Aims: 1. To create an administrative and leadership structure of the Lassa ICIDR that will: Foster effective interactions among the investigators and institutions to ensure a productive research effort, and Organize and oversee the overall fiscal management and allocation of resources that includes a Staffing Plan; 2. To organize and foster communication between the Lassa ICIDR scientists as well as external partners, including reporting to NIAID; 3. To organize meetings of the Lassa ICIDR investigators and other key personnel; 4. To identify and resolve problems and unexpected outcomes; 5. To coordinate and oversee data collection, transfer, and statistical analyses via a data management system, managed at the foreign site; 6. To oversee and assure quality control, biosafety, biocontainment, and biosecurity in laboratory practice and data management; 7. To coordinate periodic evaluation of the Lassa ICIDR, including the participation of Internal and External Advisory Groups; 8. To organize and oversee the analysis, presentation and publication of scientific data; 9. To manage the sharing of data and resources with external investigators and the management of intellectual property; and 10. To foster infrastructure development, training and education on detection, prevention, amelioration, and treatment of Lassa fever targeting both the scientific and general communities. The Core will sponsor a yearly symposium on Lassa fever, create a website, and support relevant training of laboratory and clinical staff and other investigators.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Robert Middle Name F Last Name*: Garry Suffix: Ph.D

Position/Title*:
Organization Name*: Tulane University
Department:
Division:
Street1*: 1430 Tulane Avenue
Street2: DEPARTMENT OF MICROBIOL/IMMUNOLOGY
City*: NEW ORLEANS
County:
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 701120000

Phone Number*: (504) 988-2027 Fax Number: (504) 988-1994 E-Mail*: rfgarry@tulane.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Co-PD/PI Other Project Role Category:

Degree Type: PHD,BS Degree Year: 1978, 1974

File Name

Attach Biographical Sketch*:

Attach Current & Pending Support:

Tracking Number: GRANT11602615 Funding Opportunity Number: RFA-AI-14-002 . Received Date: 2014-03-07T14:26:44.000-05:00

PROFILE - Senior/Key Person

Prefix: First Name*: Shiek Middle Name Humarr Last Name*: Khan Suffix: M.D.

Position/Title*: Director
Organization Name*: Kenema Givernment Hspital
Department: Lassa Fever Program
Division:
Street1*: 1 Combema Road
Street2:
City*: Kenema
County:
State*:
Province:
Country*: SLE: SIERRA LEONE
Zip / Postal Code*:

Phone Number*: 232 78 627 621 Fax Number: E-Mail*: Personal Info

Credential, e.g., agency login:

Project Role*: Co-PD/PI Other Project Role Category:

Degree Type: Degree Year:

	File Name
Attach Biographical Sketch*:	biosketch_of_Humarr_Khan_final.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Luis Middle Name M. Last Name*: Branco Suffix: Ph.D

Position/Title*: Co-Founder
Organization Name*: Zalgen Labs
Department:
Division:
Street1*: 20271 Goldenrod Lane, Suite 2083
Street2:
City*: Germantown
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 208760000

Phone Number*: 5044447047 Fax Number: E-Mail*: lbranco@zalgenlabs.com

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Other (Specify) Other Project Role Category: External Advisory Group

Degree Type: PHD,BS Degree Year: 2010, 1995

	File Name
Attach Biographical Sketch*:	Branco_biosketch_ICIDR.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Kathleen Middle Name A. Last Name*: Cashman Suffix: Ph.D

Position/Title*: Investigator
Organization Name*: USAMRIID
Department: Geneva Foundation
Division: Virology Division
Street1*: 1425 Porter St.
Street2:
City*: Fort Detrick
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 217020000

Phone Number*: 3016194106 Fax Number: E-Mail*: kathleen.a.cashman.ctr@mail.mil

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: External Advisory Group

Degree Type: PhD Degree Year: 2005

	File Name
Attach Biographical Sketch*:	biosketch_Cashman_28_Feb_14.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Jack Middle Name H Last Name*: Nunberg Suffix: Ph.D

Position/Title*: Director and Professor
Organization Name*: University of Montana
Department:
Division:
Street1*: Montana Biotechnology Center
Street2: Science Complex Room 221
City*: MISSOULA
County:
State*: MT: Montana
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 598120000

Phone Number*: (406) 243-6421 Fax Number: (406) 243-6425 E-Mail*: jack.nunberg@umontana.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Other (Specify) Other Project Role Category: External Advisory Group

Degree Type: PHD,AB Degree Year: 1979, 19762

	File Name
Attach Biographical Sketch*:	Nunberg_ICIDR_bio.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Pardis Middle Name Christine Last Name*: Sabeti Suffix: M.D.

Position/Title*: Assistant Professor
Organization Name*: Harvard University
Department:
Division:
Street1*: Systems Biology/ OEB
Street2: Bauer Laboratory
City*: Cambridge
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 021380000

Phone Number*: 617.252.1190 Fax Number: 617.495.2196 E-Mail*: psabeti@oeb.harvard.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Other (Specify) Other Project Role Category: External Advisory Group

Degree Type: MD,PHD,MS,BS Degree Year: 2006, 2003, 1998, 1997

	File Name
Attach Biographical Sketch*:	Biosketch-Sabeti_icidr.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Erica Middle Name Ollmann Last Name*: Saphire Suffix: Ph.D

Position/Title*: Professor
Organization Name*: The Scripps Research Institute
Department:
Division:
Street1*: The Scripps Research Institute
Street2: Dept. of Immunology
City*: LA JOLLA
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 920370000

Phone Number*: (858) 784-8602 Fax Number: (858) 784-8218 E-Mail*: erica@scripps.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Other (Specify) Other Project Role Category: External Advisory Group

Degree Type: PHD,BA Degree Year: 2000, 1993

	File Name
Attach Biographical Sketch*:	Biosketch_OllmannSaphire.pdf
Attach Current & Pending Support:	

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Robert	F	Garry		Co-PD/PI	Institutional Base Salary	EFFORT			18,150.00	3,285.00	21,435.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **21,435.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Program Manager	EFFORT			5,125.00	1,317.00	6,442.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**6,442.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**27,877.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,000.00
2. Foreign Travel Costs	4,500.00
Total Travel Cost	**6,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	623.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	15,000.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**15,623.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**50,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	35,000.00	17,675.00
		Total Indirect Costs	**17,675.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**67,675.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Admin_Core_Budget_Justification_ICIDR_v1.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Robert	F	Garry		Co-PD/PI	Institutional Base Salary	EFFORT			18,150.00	3,285.00	21,435.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **21,435.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Program Manager	EFFORT			5,125.00	1,317.00	6,442.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**6,442.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**27,877.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,000.00
2. Foreign Travel Costs	4,500.00
Total Travel Cost	**6,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT11602615 Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	623.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	15,000.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**15,623.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**50,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	35,000.00	17,675.00
		Total Indirect Costs	**17,675.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**67,675.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Admin_Core_Budget_Justification_ICIDR_v1.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Robert	F	Garry		Co-PD/PI	Institutional Base Salary	EFFORT			18,150.00	3,285.00	21,435.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **21,435.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Program Manager	EFFORT			5,125.00	1,317.00	6,442.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**6,442.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**27,877.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,000.00
2. Foreign Travel Costs	4,500.00
Total Travel Cost	**6,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	623.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	15,000.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**15,623.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**50,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	35,000.00	17,675.00
		Total Indirect Costs	**17,675.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**67,675.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Admin_Core_Budget_Justification_ICIDR_v1.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Robert	F	Garry		Co-PD/PI	Institutional Base Salary	EFFORT			18,150.00	3,285.00	21,435.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **21,435.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Program Manager	EFFORT			5,125.00	1,317.00	6,442.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**6,442.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**27,877.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,000.00
2. Foreign Travel Costs	4,500.00
Total Travel Cost	**6,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	623.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	15,000.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**15,623.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**50,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	35,000.00	17,675.00
		Total Indirect Costs	**17,675.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**67,675.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Admin_Core_Budget_Justification_ICIDR_v1.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT11602615 Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Robert	F	Garry		Co-PD/PI	Institutional Base Salary	EFFORT			18,150.00	3,285.00	21,435.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **21,435.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Program Manager	EFFORT			5,125.00	1,317.00	6,442.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**6,442.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**27,877.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,000.00
2. Foreign Travel Costs	4,500.00
Total Travel Cost	**6,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	623.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	15,000.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**15,623.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**50,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	35,000.00	17,675.00
		Total Indirect Costs	**17,675.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**67,675.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Admin_Core_Budget_Justification_ICIDR_v1.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT11602615 Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

Senior/Key Personnel:
Robert F. Garry, Ph.D. will serve as Administrative Core Co-PD EFFORT months) and will be responsible for the day-to-day planning, initiation, implementation, conduct, monitoring, and completion of tasks identified Lassa ICIDR. Dr. Garry has managed NIH grants and contract (Cooperative Agreements) that successfully produced LASV recombinant antigen-based ELISA to LASV. Second generation LF Immunoassays are essential to achieving several Aims of the current Collaborative Program. He has demonstrated the ability to: coordinate, monitor, and manage multicomponent grant activities, including management of consortia to ensure project goals are met and timelines are kept; and effectively communicate with the project team and NIAID.

Christopher Bishop, MSW is Program Manager Assistant (PMA, EFFORT months) with excellent organizational and informational technology skills. He is in charge of all day-to-day administrative and logistical aspects of the project

Supplies:
Training materials and office supplies $623

Training is an important mission of the Administrative Core. Supplies ($500 per year) are requested to prepare training materials, which will be distributed to KGH investigators and staff. A budget ($123) for office supplies is also requested.

Domestic Travel: $2,000
As specified in the RFA, funds are requested for Dr. Garry to travel to the ICIDR Kick-Off Meeting within 3 months of award. In future years he will attend the Annual Programmatic Meeting.

International Travel: $4,500
Airfare to and from Sierra Leone costs approximately $2,500. In-country travel, hotel and per diem cost an additional $500 per trip. In addition excess baggage fees to transport materials to KGH average a total of $1500 per trip. We are budgeting for Dr. Garry to travel to the Sierra Leone site 1 time per year on Lassa ICIDR funds (total time for US Co-PIs in Sierra Leone exceeds RFA requirements for total US PI time spent at the International site). Dr. Garry regularly travels to Sierra Leone at least 4 times per year for a total stay of over 2 months.

Indirect Costs:
The Indirect Cost Rate for the Tulane University School of Medicine (TUHSC) is 50.5% (Modified Total Direct). Indirect costs are not applied to equipment or Consortium costs.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**107,175.00**
Section B, Other Personnel		**32,210.00**
Total Number Other Personnel	5	
Total Salary, Wages and Fringe Benefits (A+B)		**139,385.00**
Section C, Equipment		**0.00**
Section D, Travel		**32,500.00**
1. Domestic	10,000.00	
2. Foreign	22,500.00	
Section E, Participant/Trainee Support Costs		**0.00**
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		**78,115.00**
1. Materials and Supplies	3,115.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	75,000.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		**250,000.00**
Section H, Indirect Costs		**88,375.00**
Section I, Total Direct and Indirect Costs (G + H)		**338,375.00**
Section J, Fee		**0.00**

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Shiek	Humarr	Khan	M.D.	Co-PD	Institutional Base Salary	EFFORT			4,500.00	675.00	5,175.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **5,175.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Program Manager	EFFORT			3,000.00	450.00	3,450.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**3,450.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**8,625.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	1,500.00
Total Travel Cost	**5,500.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	875.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**875.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**15,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**15,000.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Admin_Core_KGH_Budget_Justification_ICIDR.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Shiek	Humarr	Khan	M.D.	Co-PD	Institutional Base Salary	EFFORT			4,500.00	675.00	5,175.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **5,175.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Program Manager	EFFORT			3,000.00	450.00	3,450.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**3,450.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**8,625.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	1,500.00
Total Travel Cost	**5,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	875.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**875.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**15,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**15,000.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Admin_Core_KGH_Budget_Justification_ICIDR.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 3**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1. Dr.	Shiek	Humarr	Khan	M.D.	Co-PD	Institutional Base Salary	EFFORT			4,500.00	675.00	5,175.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **5,175.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Program Manager	EFFORT			3,000.00	450.00	3,450.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**3,450.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**8,625.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	1,500.00
Total Travel Cost	**5,500.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	875.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**875.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**15,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**15,000.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Admin_Core_KGH_Budget_Justification_ICIDR.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Shiek	Humarr	Khan	M.D.	Co-PD	Institutional Base Salary	EFFORT			4,500.00	675.00	5,175.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **5,175.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Program Manager	EFFORT			3,000.00	450.00	3,450.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**3,450.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**8,625.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	1,500.00
Total Travel Cost	**5,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	875.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**875.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**15,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**15,000.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Admin_Core_KGH_Budget_Justification_ICIDR.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Shiek	Humarr	Khan	M.D.	Co-PD	Institutional Base Salary	EFFORT			4,500.00	675.00	5,175.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **5,175.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Program Manager	EFFORT			3,000.00	450.00	3,450.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**3,450.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**8,625.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	1,500.00
Total Travel Cost	**5,500.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	875.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**875.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**15,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**15,000.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Admin_Core_KGH_Budget_Justification_ICIDR.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Senior/Key Personnel:
Sheik Humarr Khan, M.B.Ch.B., will serve as Administrative Core Co-PD EFFORT months) and will be responsible for the day-to-day planning, initiation, implementation, conduct, monitoring, and completion of tasks identified Lassa ICIDR. Dr. Khan will also serve as overall Co-PI of the Lassa ICIDR. He is Director of the National Lassa Fever Program of the Ministry of Health and Sanitation (MOHS) in Sierra Leone. He is permanently assigned to Kenema Government Hospital. He has over 10 years experience treating Lassa patients and recently completed a residency in Internal Medicine at Korle Bu Teaching Hospital in Ghana Dr. Khan is responsible for patient admission, testing and medical care on the Lassa Fever Ward. As Director of the National Lassa Fever Program, he also oversees activities of the Lassa Fever Laboratory and Outreach Teams.

Simbirie Jalloh will serve as Program Coordinator (PMA, EFFORT months). Ms. Jalloh has 5.5 years experience and excellent organizational and informational technology skills. She will be responsible for all day-to-day administrative and logistical aspects of the managing the project in Sierra Leone. Ms. Jalloh has visited the US twice, meeting with NIH program officers, contracting officers and other budgetary officials. She has received training from and works closely with investigators, program managers and grants and Contracts personnel at Tulane University and other subcontractors. Ms. Jalloh will manage the budget, keep financial and other records. She will arrange transport and accommodation for visiting scientists.

Supplies:
Training materials and office supplies $875

Training is an important mission of the Administrative Core. Supplies ($375 per year) are requested to prepare training materials, which will be distributed to KGH investigators and staff. A budget ($500) for general office supplies is also requested.

Travel from Sierra Leone to the United States): $4,000
Airfare to and from Sierra Leone costs approximately $2,500. In-country travel, hotel and per diem cost an additional $500 per trip. As specified in the RFA, funds are requested for Dr. Khan to travel to the ICIDR Kick-Off Meeting within 3 months of award. In future years he will attend the Annual Programmatic Meeting.

Travel within Sierra Leone: $1,500
Dr. Khan will visit each of the field sites at least once per year. He also will meet regularly with the central MOHS to inform them of Lassa ICIDR progress and results.

Indirect Costs:
None requested

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**25,875.00**
Section B, Other Personnel		**17,250.00**
Total Number Other Personnel	5	
Total Salary, Wages and Fringe Benefits (A+B)		**43,125.00**
Section C, Equipment		**0.00**
Section D, Travel		**27,500.00**
1. Domestic	20,000.00	
2. Foreign	7,500.00	
Section E, Participant/Trainee Support Costs		**0.00**
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		**4,375.00**
1. Materials and Supplies	4,375.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		**75,000.00**
Section H, Indirect Costs		**0.00**
Section I, Total Direct and Indirect Costs (G + H)		**75,000.00**
Section J, Fee		**0.00**

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OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:	Dr.
First Name*:	Robert
Middle Name:	F
Last Name*:	Garry
Suffix:	Ph.D

2. Human Subjects

Clinical Trial?	● No	○ Yes
Agency-Defined Phase III Clinical Trial?*	○ No	○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ○ No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

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5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	ADMIN_CORE_ICIDR_Specifc_Aims.pdf
3. Research Strategy*	ICIDR_Admin_Core_final.pdf
4. Progress Report Publication List	Progress_Report_Publication_List.pdf
Human Subjects Sections	
5. Protection of Human Subjects	Admin_Core_no_Human_Subjects.pdf
6. Inclusion of Women and Minorities	Admin_Core_-_Women.pdf
7. Inclusion of Children	Admin_-_Children.pdf
Other Research Plan Sections	
8. Vertebrate Animals	Admin_Core_no_Vert_Animals.pdf
9. Select Agent Research	SELECT_AGENT_RESEARCH_ICIDR.pdf
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	CONSORTIUM_AGREEMENT_ICIDR.pdf
12. Letters of Support	LOS_Bundle_final.pdf
13. Resource Sharing Plan(s)	RESOURCE_SHARING_ICIDR.pdf
Appendix (if applicable)	
14. Appendix	

International Collaboration in Infectious Disease Research on Lassa fever (Lassa ICIDR)

SPECIFIC AIMS (ADMINISTRATIVE CORE)

An efficient administrative structure is essential to the design and implementation of this U19 Program. The Lassa ICIDR Administrative Core is designed to facilitate communication, planning, data sharing, and scientific and fiscal oversight of the component research projects of the Lassa ICIDR. Through the Administrative Core will oversee all the goals of Lassa ICIDR Program. The Core will fund the annual travel of the External Advisory Board to the Program site of review. The Core Program Managers will coordinate, schedule, and implement minimum monthly internal meetings and teleconferencing among the Program sites, and will assist the Co-PDs of the Projects in the preparation of progress reports, internal compliance filings and edit manuscripts to support the Program. The Core will oversee data sharing, data archiving, and overall record keeping of the Program.

The Administrative Core will be responsible for the overall organization, management, decision-making, and evaluation of the Lassa ICIDR with the following Specific Aims:

1. To create an administrative and leadership structure of the Lassa ICIDR that will:
    a. Foster effective interactions among the investigators and institutions to ensure a productive research effort, and
    b. Organize and oversee the overall fiscal management and allocation of resources that includes a Staffing Plan
2. To organize and foster communication between the Lassa ICIDR scientists as well as external partners, including reporting to NIAID;

3. To organize meetings of the Lassa ICIDR investigators and other key personnel;

4. To identify and resolve problems and unexpected outcomes;

5. To coordinate and oversee data collection, transfer, and statistical analyses via a data management system, managed at the foreign site;

6. To oversee and assure quality control, biosafety, biocontainment, and biosecurity in laboratory practice and data management;

7. To coordinate periodic evaluation of the Lassa ICIDR, including the participation of Internal and External Advisory Groups;

8. To organize and oversee the analysis, presentation and publication of scientific data;

9. To manage the sharing of data and resources with external investigators and the management of intellectual property; and

10. To foster infrastructure development, training and education on detection, prevention, amelioration, and treatment of Lassa fever targeting both the scientific and general communities. The Core will sponsor a yearly symposium on Lassa fever, create a website, and support relevant training of laboratory and clinical staff and other investigators.

RESEARCH STRATEGY (ADMINISTRATIVE CORE)

The Lassa ICICR will enhance and utilize the symmetrical and highly productive partnership developed over the last decade between Tulane University and the Lassa fever Program at Kenema Government Hospital (KGH). We believe that the KGH Lassa fever research program can with the proper training and commitment perform human clinical trials of Lassa fever diagnostics, therapeutics, immunotherapeutics and vaccines, now in advanced stages of development through NIAID supported research projects. After nearly forty years of research on Lassa fever, there is now great promise for controlling, treating or preventing this devastating disease, which is of major importance to Sierra Leone. Therefore, as an over-riding theme, the Lassa ICIDR program will promote the development of research capacity at KGH, with a particular emphasis on training Sierra Leonean research staff. The program will also enhance relevant scientific linkages between United States and KGH LF investigators, and encourage future collaborative relationships with other research groups leading to improvements in detection, prevention, amelioration, and treatment of Lassa fever in the subregion. The Administrative Core will provide the required administrative infrastructure and scientific leadership to meet these goals. The organizational capacity of the Core will also provide necessary strategic planning, implementation, integration, communication, and monitoring.

Specific Aim 1: To create an administrative and leadership structure of the Lassa ICIDR.

The Lassa ICIDR and its management structure is organized to form a cohesive, integrated and efficient unit that provides scientific and administrative oversight of the Research Projects and Core (Fig. 1). To create an administrative and leadership structure of the Lassa ICIDR that will:

a) Foster effective interactions among the investigators and institutions to ensure a productive research effort, and
b) Organize and oversee the overall fiscal management and allocation of resources that includes a Staffing Plan (Fig. 2).

The Administrative Core leadership will consist of the following team:

Sheik Humarr Khan, MBCHB, MWACP (Administrative Core Co-Director, Overall ICIDR Co-PI) is one of the world's leading experts in the clinical care of patients with viral hemorrhagic fevers. He is a product of the College Of Medicine and Allied Health Sciences (COMAHS) of University of Sierra Leone (USL). After graduating with a Bachelor's of Medicine and Bachelors in Surgery (MBChB) in 2001, Dr. Khan was recruited as a medical officer at the Directorate of Disease Prevention and Control, Ministry Of Health and Sanitation (MOHS) where he served until 2005. Dr. Khan stepped into the shoes of his predecessor, the late Dr. Aniru Conteh who unfortunately died of the Lassa fever. In his capacity as the Chief Physician of the Lassa Fever Program, KGH, Dr. Khan was concurrently contracted by then United Nations Mission in Sierra Leone (UNAMSIL) as a physician and consultant for Lassa fever in Sierra Leone, as Physician In-charge of HIV/AIDS services, KGH, and as Physician Consultant, Mano River Union Lassa fever network, WHO/Tulane University. Dr. Khan was awarded the degree of MWACP after residency training in the field internal medicine at the Korle-Bu Teaching Hospital (KBTH), Accra, Ghana (2013). He is now back to his field of work as the In-Charge of the Lassa Fever Program, KGH, MOHS In January, 2014. He is extremely well qualified to direct the Administrative Core, and the Lassa ICIDR overall.

Robert F. Garry, PhD (Administrative Core Co-Director, Overall ICIDR Co-PI) will share responsibilities with Dr. Khan for the overall supervision and coordination of all Lassa ICIDR activities via the Administrative Core. Dr. Garry is Professor of Microbiology and Immunology at Tulane University, and Assistant Dean for the Graduate Program in Biomedical Sciences. He has extensive experience overseeing and managing field research projects and investigations in sub-Saharan Africa. He is currently managing a consortium of scientists who are developing countermeasures, including diagnostics, immunotherapeutics and vaccines, against Lassa virus and several other biodefense pathogens (vhfc.org). His team has produced LASV point-of-care and confirmatory diagnostics based on recombinant proteins that have high sensitivity for detecting

infection with LASV. Dr. Garry serves in the Tulane University administration as Assistant Dean for Graduate Studies in Biomedical Sciences and is committed to the training of young scientists in the United States and abroad. He will also interact will each of the Projects and also work closely with other ICIDR scientists on the crucial training aspects of the Collaborative Center, by offering workshops seminar and by identifying Sierra Leonean candidates for external training.

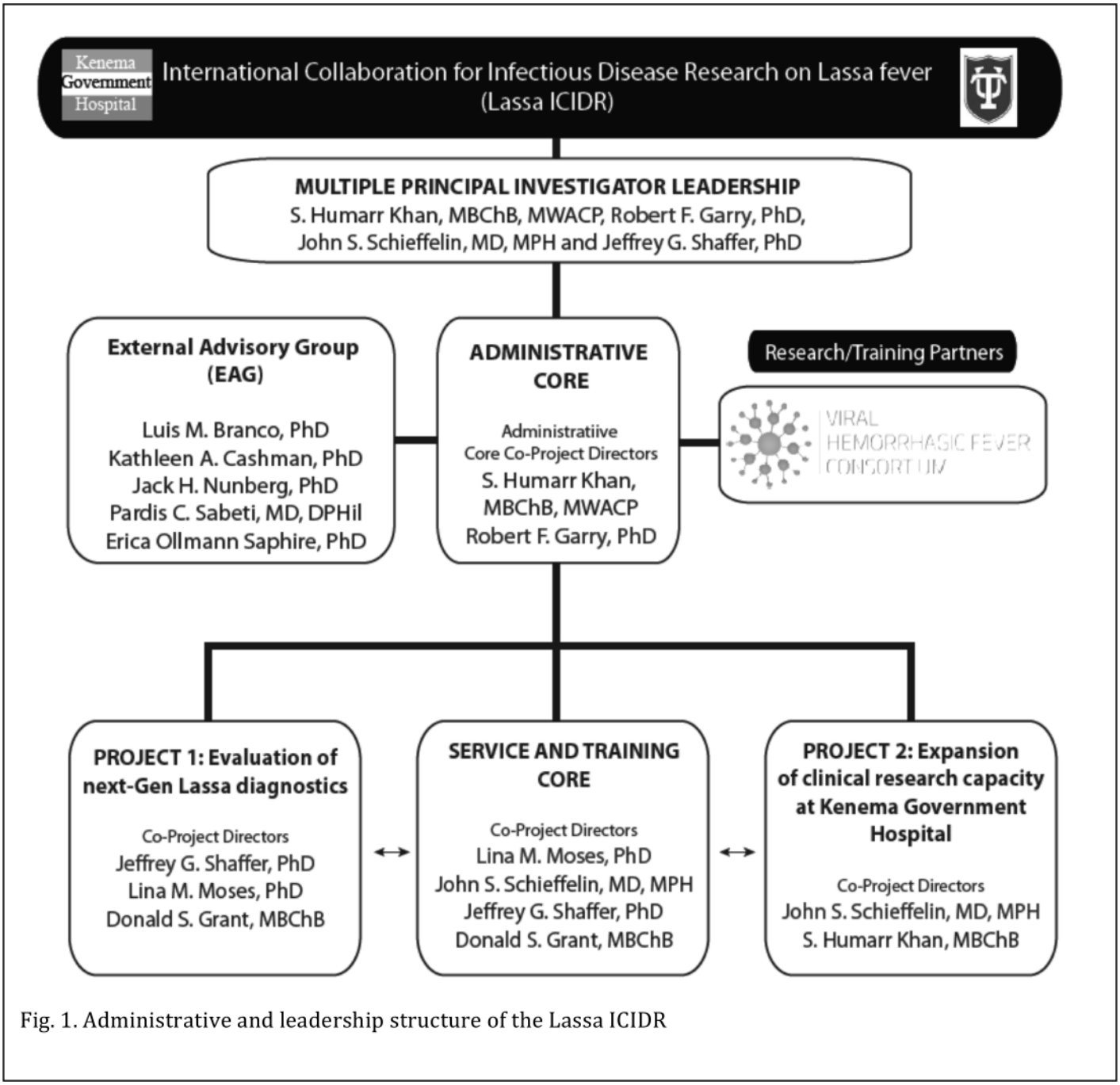


Fig. 1. Administrative and leadership structure of the Lassa ICIDR

Drs. Khan and Garry will be assisted by two talented Program Coordinators:
Simbirie Jalloh will serve as Program Coordinator at KGH reporting directly to Dr. Khan. Ms. Jalloh has 5.5 years experience and excellent organizational and informational technology skills. She will be responsible for all day-to-day administrative and logistical aspects of the managing the project in Sierra Leone. Ms. Jalloh has visited the US twice, meeting with NIH program officers, contracting officers and other budgetary officials. She has received training from and works closely with investigators, program managers and grants

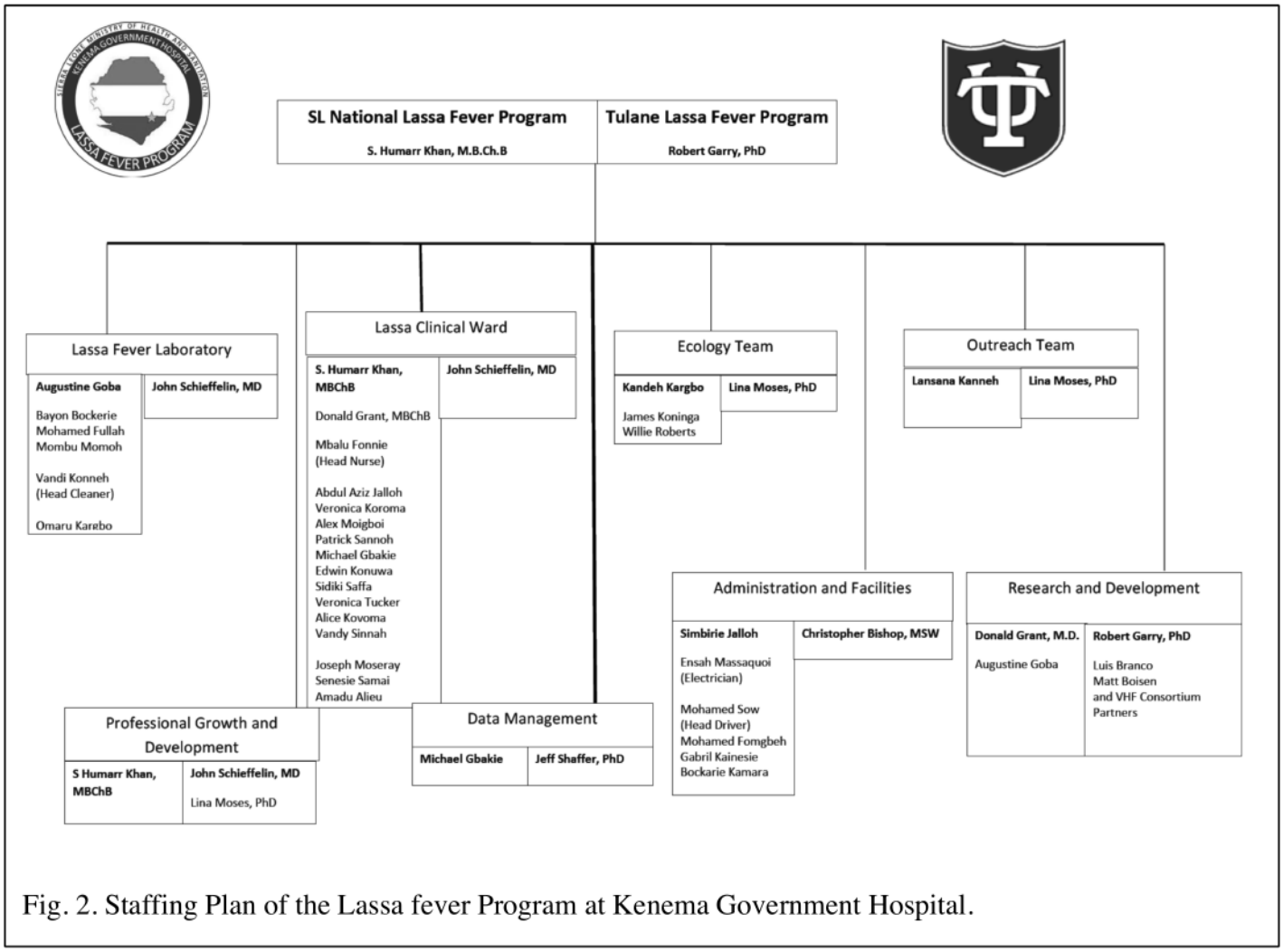


Fig. 2. Staffing Plan of the Lassa fever Program at Kenema Government Hospital.

and Contracts personnel at Tulane University and other subcontractors. Ms. Jalloh will manage the budget, keep financial and other records. She will arrange transport and accommodation for visiting scientists.

Christopher Bishop, a Program Coordinator with excellent organizational and informational technology will be based at Tulane and will manage the budget, keep financial and other records. Tulane will provide the core administrative systems and staff to assist the PIDs and PMs in conducting the financial management and reporting activities of the consortium, which will include contract cost tracking, analysis of consortium cost reimbursement requests, appropriate disbursement of funds, and other related financial management responsibilities. Tulane will follow approved university procedures for processing purchases, consortium invoices, and other expense items. In addition, the Tulane University Offices of Sponsored Projects Administration and will be responsible for reviewing and approving expenses to ensure compliance with federal regulations, for issuing consortium agreements between Tulane University and KGH, for establishing sponsored accounts in the Tulane Accounting System, and for creating invoices and financial reports based on project expenses.

Staffing Plan

All divisions of the Lassa ICIDR staff have established infrastructure. There will be designated individuals (highlighted in BOLD in Figure 2), who will serve as the primary contacts with the PDs and PMs. In addition to their scientific and technical contributions to achieving Project Requirements and Milestones, the responsibilities of these individuals will be to manage timelines and coordinate activities to insure completion of the projects within the allotted time; participate in and coordinate weekly and monthly teleconferences

between ICIDR members; provide monthly summary reports to the PIs; provide additional technical and regulatory reports as required; review data and reports for scientific content, interpretation, and consistency; coordinate regulatory audits and site visits; and resolve any issues that may arise during the conduct of the proposed projects.

Institutional Support
Both the federal government of Sierra Leone and Kenema Government Hospital have consented to provide full institutional support of this proposal. This application includes letters from Dr. Christian Partt, Director of KGH, and Dr. Brima Kargbo, Chief Medical Officer of the MOHS of Sierra Leone. These letters describe the strong commitment of KGH and the Government of Sierra Leone to implement the Lassa ICIDR at KGH, and participate and sustain this important international resource.

Tulane University has a long history of participation in international research and training programs. Along with the full support of Program Co-PI Dr. Garry, L Lee. Hamm, MD, Senior Vice-President and Dean of the School of Medicine has provided a strong letter committing support to the Lassa ICIDR.

Specific Aim 2: To organize and foster communication between the Lassa ICIDR scientists as well as external partners, including reporting to NIAID.
Communication between the investigators and support personnel of the Projects and Cores will be essential to the success of the Lassa ICIDR. Although a formal system of communications will be implemented (see below), as is commonplace, regular informal contacts by email and phone will constitute the first line and probably most important element of the communication network. Communication will be facilitated by the fact that, although this is an original proposal, most of the scientists involved in this Collaborative Program already know each other well and have a history of working together and communicating regularly on an regular basis. The Co-PIs will continue and intensify this regular phone/email communication with the Project/Core Leaders on a roughly weekly basis to review scientific progress and administrative issues, and to discuss and resolve any obstacles to progress that may arise. Issues that require the input of the larger Lassa ICIDR team will be placed on the agenda for the biweekly meeting of the Administrative Core or the monthly teleconferences of all key Project personnel (see below). When necessary, issues will also be referred to the members of the internal advisory group (IAG) and/or EAG (see below).

Establishment of a formal communications network will be facilitated by the existing network in place for Tulane research activities as well as the formal Viral Hemorrhagic Fever Consortium (vhfc.org) in West Africa. For example, monthly research and patient care reports are already produced by the KGH Lassa Laboratory and Ward and sent to Drs. Khan. Garry, Schieffelin (Clinical Coordinator) and Shaffer (Data Manger/Statistician). Among other communication responsibilities, the Administrative Core will coordinate all scientific, regulatory, and financial reporting to NIAID, including progress in meeting Project Milestones and annual reports. Project/Core leaders will be required to submit brief (1-2 pages) quarterly reports summarizing activities and results from their units. These will be compiled by the Co-PIs and used, in part, as the basis of Milestone reports to NIAID. In addition to the scientific and technical contributions required to achieve project Milestones and resolve any issues that may arise during the conduct of the proposed project. With participants in Sierra Leone and the United States, adherence to these principles is absolutely necessary for the successful completion of project aims and the creation of a sustainable research program in West Africa.

Specific Aim 3: To organize meetings of the Lassa ICIDR investigators and other key personnel
Kick-off meeting with NIAID. The Lassa ICIDR will send representatives to the RFA required ICIDR Kick-off meeting in the Washington DC area. Co-PIs, Drs. Khan, Garry Schieffelin and Shaffer will attend.

Biweekly Meetings of Project/Core Staff Meetings. Project and Core leaders will be responsible for frequent communication with their co-investigators and the members of their laboratories and units. Regular (weekly

or biweekly) meetings of the personnel at each Project or Core site will be organized by the Project/Core Leader to review research activities and results and to identify and propose solutions to any potential problems. In almost all cases, matters related to this Project Program can simply be added on to the agendas of existing laboratory meetings. Issues that require input or review from the broader Lassa ICIDR team will be added to the agenda for the monthly teleconference.

Monthly Teleconferences. A monthly teleconference with all Project/Core Leaders as well as other key personnel will be scheduled. An agenda will be prepared in advance covering operational issues and updates on the status of data analysis, manuscript preparation, and outside collaborations. Each Project/Core Leader will be expected to give a status report. To maximize efficiency, the teleconference will be held just after one of the aforementioned biweekly meetings of the *Project/Core Staff*. Minutes will be taken by the Program Coordinator and distributed by email to all Lassa ICIDR Project/Core Leaders and other key personnel within 48 hours of the teleconference. Material from these minutes will also be extracted to be included in formal reports to NIAID.

Annual Investigators Meetings. A one-day meeting of all Lassa ICIDR investigators and other key personnel will be held annually, with an agenda prepared in advance. The meeting attendance is estimated to be approximately 30 persons, including members of the IAC and EAG. To simplify travel plans for our colleagues in Sierra Leone, when held in the United States we will schedule these meetings to take place in conjunction with the Annual Meeting of the American Society of Tropical Medicine and Hygiene (ASTMH) or another scientific meeting. This will afford the opportunity for Project personnel to enhance their education by attending the meeting, and to facilitate communication of results from the Project via posters and presentations.

Specific Aim 4: To identify and resolve problems and unexpected outcomes

Given the history of working together and excellent rapport between the investigators of the Lassa ICIDR, we anticipate the vast majority (if not all) of any problems that might arise to be resolved quickly through one-to-one discussions. Problems that require the input of the larger Lassa ICIDR team will be placed on the agenda for discussion at the biweekly meeting of the Administrative Core and/or the monthly teleconferences. Although unanticipated, if problems persist, they can be referred to the members of the internal advisory committee and/or EAG. The Program Director will have the responsibility of guiding the team to achieve consensus on any issues, and will ultimately make the necessary decisions and chart the direction of the Lassa ICIDR.

Specific Aim 5: To coordinate and oversee data collection, transfer, and statistical analyses

Efficient analysis, transfer, integration, monitoring, and quality control of data collected from the various Projects/Cores will be essential to the Lassa ICIDR's success and will be the overall responsibility of the Administrative Core.

Data collection and linkage. For all ongoing research projects at the KGH site, the human subject data collection process is outlined as follows:

1. Each incoming subject is assigned a unique identification numbers that is sequentially logged in a logbook.
2. For subjects interested in participating in a study, a signed informed consent form is obtained.
3. A paper-based pre-admission evaluation form is completed.
4. Blood samples are drawn using tubes labeled with their identification numbers.
5. Blood samples are tested for Lassa fever using ELISA, PCR, and rapid diagnostic methods. Raw laboratory data are generated in digital format.
6. Laboratory results are recorded on a paper-based form and are used to determine whether patients are admitted to the hospital.

7. If patient is admitted to the hospital, additional clinical data are collected and maintained using a series of paper-based clinical forms.
8. The pre-admission evaluation form, clinical forms, and laboratory results forms are forwarded to a KGH employee for entry into a relational database, excluding all personally identifiable information.
9. All paper-based forms are scanned and redacted of all personally identifiable information.
10. The database, scanned forms, and raw laboratory data are made available to Tulane personnel via a secure file sharing application.

All data are collected and logged on a continuous basis by KGH staff. Tulane personnel are responsible for revising the relational database and providing training on its use. Each of the digital forms are named using the appropriate patient identification number, and the collection of digital forms are constitutes a patient's digital medical chart. Due to the different time points associated with patient survival outcome, admission status, and ribavirin treatment, these data are determined via comprehensive medical chart and log book reviews. These chart reviews are conducted using the digital medical charts, and the logbook reviews are conducted during annual on-site data audits. Consent forms are reviewed and their identification numbers are logged into a spreadsheet to provide consent information absent of personally identifiable information.

Lassa laboratory data are maintained separately and consist of results from Lassa fever diagnostics and blood chemistry analyses. The Lassa diagnostics include antigen (Ag) and immunoglobulins M and G (IgM, and IgG, respectively) ELISA approaches. Rapid diagnostic and polymerase chain reaction (PCR) methods are also used. The Ag, IgM, and IgG results are generated using the Visual FoxPro ELISA plate reader software in Microsoft Excel format and are digitally archived and transcribed into a paperbound laboratory notebook. Blood chemistry data are generated using a Piccolo® Clinical Chemistry Analyzer and are archived electronically. All of the laboratory data are referenced by patient identification number, blood draw number, and plate or read number. The electronic files housing the raw data are standardized and compiled using a suite of Visual Basic macros. The PCR results are maintained separately on a paper based form, which is redacted and logged into a relational database.

All available data sources are linked using the SAS System (version 9.3; SAS Institute, Cary, NC.). The primary matching variable is the unique patient identification number. A single algorithm is written to combine and pool the data over all of the sources. The raw laboratory results are compared to the paper-based laboratory results for accuracy, and the raw laboratory results are considered as the standard for publication purposes. The data are progressively cleaned and curated via regular on-and off-site data audits, where data are checked against their paper-based questionnaire forms and laboratory notebooks. Data are backed up onto a cloud storage system and made available to key personnel. Data verification techniques include source data verification using the scanned medical charts, logic checks, range checks, and consistency checks. Data validation is also maintained through the aforementioned relational database using electronic forms, input masks and validation rules.

Data confidentiality and storage. Data collected at the Kenema site includes completed paper forms, electronic text, electronic images, and blood samples. All identifiable data for our ongoing studies for the Kenema site are maintained at the KGH. Tulane and KGH personnel are responsible ensuring the de-identification of data prior to using the data for verification or analysis at any other site. Publications or reports using data collected at the KGH site are only reported or published in in aggregate form with generalized results. All paper forms, electronic images, and blood samples are maintained by KGH personnel and are not transmitted to any other location. The blood samples are stored at the KGH site in a laboratory with restricted access for only authorized Tulane and KGH staff. The limited data maintained at Tulane include the relational database, scanned forms, the consent log spreadsheet, and raw laboratory data, all of which are de-identified prior to they are made available. The relational database and scanned forms are transmitted to the Tulane location electronically using a secure file sharing application. All host computers for Tulane personnel with access to the study data are password-protected and equipped with approved firewalls and antivirus software. Compliance to these data security safeguards is ensured by requiring all project personnel to complete the NIH Computer Security Awareness Training Course. This data collection and storage process complies with HIPAA 45 CFR part 46 and HIPPA security requirements as described in HIPPA CFR Parts 160 and Subparts A and C of Part 164.

Specific Aim 6. Oversee and assure quality control, biosafety, biocontainment, and biosecurity in laboratory practice and data management.
Standard Operation Procedures exist for all assays presently being performed in the Project and Core laboratories. All laboratory assays have internal positive and negative controls incorporated into each run, and are performed in duplicate when feasible. New assays being implemented in the LASSA ICIDR will be optimized, standardized, and validated on known positive and negative controls in the specific laboratory where the assay is to be performed by investigators familiar with the procedure. This will be especially pertinent for implementation of new assays in Kenema. For training purposes, the investigator and local laboratory technician will work together on the optimization, standardization, and validation and, through the process, create Standard Operating Procedures for the specific laboratory. Quality control will then continue to be monitored through a variety of methods. Project and Core leaders will have primary responsibility for monitoring data quality in their respective laboratories and units.

BIOSAFETY, BIOCONTAINMENT, AND BIOSECURITY
Biosafety, biocontainment, and biosecurity policies and regulations are presently in place in all Lassa ICIDR Project and Core laboratories. These will be reviewed by the Lassa ICIDR Administrative Core in collaboration with the IAG and EAG and strictly reinforced where needed. Specific details to approaches to biosafety, biocontainment, and biosecurity are provided in the individual Project and Core proposals.

Spe**cific Aim 7: Coordinate periodic evaluation of the Lassa ICIDR, including the participation of internal and External Advisory Groups.**

Internal Advisory Committee. Drs. Khan and Garry will be assisted in execution of both the administrative and scientific research elements of the Project Program by an IAC consisting of the leaders of each individual Project/Core and selected other key personnel working on the Project. The Committee will convene monthly in person or by teleconference as detailed above under *Communications*.

John S. Schieffelin, MD, MSPH is an Assistant Professor of Pediatrics and Internal Medicine at Tulane University and is trained in pediatrics, internal medicine, infectious diseases, tropical medicine and public health. He has extensive experience overseeing and managing field research projects and investigations in Sierra Leone. He is well versed in the laboratory diagnosis of VHFs. In addition, Dr. Schieffelin has extensive experience generating and analyzing the function of human monoclonal antibodies against multiple viral pathogens including dengue virus and Lassa virus. For the past three years, Dr. Schieffelin has been the Clinical Director of the Viral Hemorrhagic Fever Consortium. In this role, he has overseen all human subjects research conducted at the Kenema Government Hospital LF Ward through three large federally funded research programs. He supervises all subject recruitment, clinical specimen and data collection on the Lassa Fever Ward as well as specimen processing and analysis in the Lassa Fever Laboratory. He oversees the activities of over 30 Tulane-affiliated collaborators.

Jeffery G. Shaffer, PhD is an Assistant Professor at Tulane's School of Public Health and Tropical Medicine and has extensive experience in the management and statistical analysis of health and clinical data, specifically for diseases in sub-Saharan Africa, environmental exposures, breast and prostate cancers, and infant mortality. Dr. Shaffer's statistical expertise spans database management, experimental design, statistical programming, sample size and statistical power assessment, geographic information systems (GIS), multilevel modeling, trend analysis, survey design and analysis, and PDA-based surveys. He has a sound understanding of underlying theoretical aspects statistical applications as evidenced in his article for laying out statistically optimal mating experiments. Dr. Shaffer also has considerable experience developing training workshops, where he developed a workshop for analyzing time trends and delivered it at several national conferences. Since 2010, he has served as a co-investigator for the Viral Hemorrhagic Fever Consortium (VHFC; www.vhfc.org).

Lina M. Moses, PhD is a Research Scientist in the Department of Microbiology and Immunology at Tulane Medical School. Her primary interest is in the disease ecology of viral zoonoses, utilizing population biology, ecology and epidemiological methods to understand macro-level pathogen/host interactions and monitor reservoir host populations. The ultimate goal of her research is to develop community-level interventions to halt animal to human transmission. Moses has been involved in Tulane's Lassa Fever Program since 2006 and currently directs community and ecology-based research projects. She has overseen district-wide serosurveys, community prevention interventions, case investigations and contact tracing, and observational epidemiological and ecological studies partnered with the Kenema Government Hospital's ecology and community outreach teams. Moses received her PhD from Tulane University's School of Public Health and Tropical Medicine with transdisciplinary training in community health, environmental health and tropical medicine in 2012.

Donald S. Grant, MBChB is the deputy medical director for the Ministry of Health & Sanitation Lassa fever Program based at KGH in eastern Sierra Leone. Kenema Government Hospital hosts the only referral ward and laboratory for the diagnosis and treatment of Lassa fever in the Mano River Union region of West Africa and Dr. Grant is its sole physician. In addition to his clinical responsibilities, he am also the key liaison and collaborator Tulane, Broad Institute and Harvard on biomedical and public health aspects of Lassa fever. The proposed study will have a direct and immediate impact on disease prevention, laboratory diagnosis and treatment for individual patients and affected communities in Sierra Leone. Dr. Grant will coordinate \with the experienced team in Sierra Leone, including Mr. Augustine Goba, Director of the KGH Lassa Laboratory, Mr. Lansana Kanneh, Supervisor of the Outreach Team, which conducts all patient follow-up, visits as well as the Mbalu Fonnie and numerous nurses, technicians and support staff. This team has been working together on Tulane Lassa Fever projects for over 10 years. Some members have over 30 years experience working with Lassa fever. It is undoubtedly the most experienced team of clinical and laboratory LASV researchers in the world.

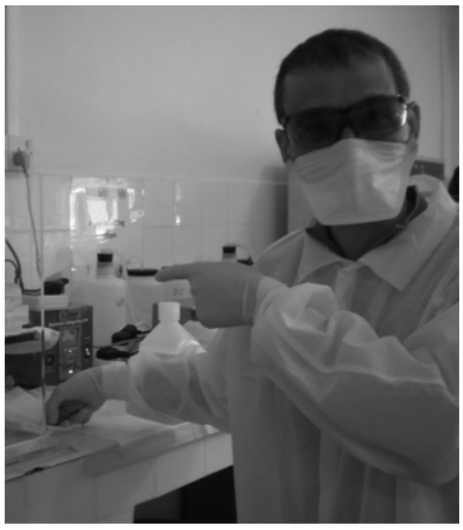


Figure 3. External Advisory Group member Luis Branco, PhD (Zalgen Labs, Germantown Maryland) in the Lassa Laboratory at KGH.

External Scientific Advisory Group. Outside experts in the immunology, pathology, diagnosis, and clinical presentation/management of Lassa fever will serve on the Project's EAG. The EAG is intended to provide yearly objective evaluation of the scientific progress as well as operational aspects of the Project Program and guide it for the next project year. EAG members will be invited to attend the annual investigators meeting. Travel arrangements will be made by the Program Coordinator and paid for through the Lassa ICIDR. After the meeting, the Program Co-Director will meet with the advisors to solicit recommendations for the research program. When considered appropriate, EAG members may also occasionally be invited to participate in the monthly teleconferences.

The members of the EAG were selected based on their experience with cutting edge Lassa fever diagnostics, therapeutics, immunotherapeutics and vaccines. This is in keeping with the goal of enabling the Lassa fever program to conduct clinical trials of means to controlling, treating or prevent Lassa fever.

Luis M. Branco, PhD, Co-Founder Zalgen Labs has extensive expertise in multiple facets of research and development of chimeric, humanized, human, and affinity maturation of recombinant antibodies for human use (Fig. 3). He as also developed extensive experience in expression of recombinant antigens in a multitude of systems, namely bacterial, baculoviral, and mammalian backgrounds over 24 years, including conceptual design, development, and patenting of a novel, regulatory compliant, and superior mammalian cell-based expression platform for generation of commercial grade proteins (U.S.

Figure 4. External Advisory Group member Kat Cashman in the BSL-4 suite at USAMRIID.

Patent US8076102). During his 17 years in industry he have been directly involved in the development of licensed antibody therapeutics, such as MedImmune's Synagis (Palivizumab), Human Genome Sciences' Benlysta (Belimumab) and ABthrax (raxibacumab), as well as additional antibodies under clinical evaluation (MEDI's Numax [motavizumab], HGS' CCR5mAb004, HGSETR1 [Mapatumumab]. His research interests in industry also focused on development of industry leading technologies for acceleration of stable cell line development with high regulatory compliance, aimed at reducing timelines and costs in the development path toward IND filings.

Kathleen A. Cashman, PhD, Investigator, Geneva Foundation, Virology Division, USAMRIID Since joining USAMRIID in 2005, she has been engaged in the development and testing of DNA-based vaccines against arenavirus infections and the development and characterization of the animal models supporting the vaccine research endeavors (Fig. 4). At USAMRIID she is currently engineering, refining and testing a DNA vaccine that has been shown to be 100% protective in both guinea pig and primate models of Lassa fever. This vaccine is moving forward for further development with the end goal of preparing the vaccine for use in humans. Dr. Cashman has over eight years of experience working in a biosafety level 4 (BSL-4) laboratory. She has direct experience designing, coordinating and conducting both *in vitro* and *in vivo* vaccine and therapeutic efficacy studies in the guinea pig and nonhuman primate models of arenavirus hemorrhagic fever. She has also been involved in fruitful collaborations with industry and academic partners for basic research, therapeutics development and efficacy studies, and vaccine development.

Figure 5. Jack Nunberg University of Montana

Jack H. Nunberg, PhD, Professor, University of Montana. Dr. Nunberg's career includes biotechnology/pharmaceutical R&D and, more recently, academia (Fig. 5). He has been at The University of Montana since 1996 as Director of the Montana Biotechnology Center and Professor of Biological Sciences. His laboratory focuses on virus entry and its inhibition, initially with HIV-1 and for the past 9 years with the arenavirus envelope glycoprotein GPC. During this latte period, we have published 19 articles that have characterized structure-function relationships that promote GPC's pH-dependent membrane-fusion activity and its inhibition by small-molecule fusion inhibitors discovered by SIGA Technologies and the Scripps Research Institute. We have also collaborated with Dr. Brian Gowen Toyama Chemical Co. to investigate the molecular basis for T-705 (favipiravir) inhibition of the arenavirus RNA-dependent RNA polymerase and identified and characterized the intracellular structures organized by arenaviruses for their replication.

Pardis C. Sabeti, M.D., D.Phil. Associate Professor at the Center for Systems Biology at Harvard University, Department of Organismic and Evolutionary Biology, and Senior Associate Member of the Broad Institute (Fig. 6). Dr. Sabeti is a computational geneticist with extensive expertise studying genetic diversity in humans and pathogens, developing algorithms to detect genetic signatures of natural selection, and carrying out genetic association studies. Dr. Sabeti has developed novel methods to detect natural selection, and applied it to the entire human genome, finding many novel candidates linked to infectious diseases. She identified candidate genes associated with natural selection for LASV infection in populations in Nigeria and Sierra Leone. Recently together with several collaborators, Drs. Sabeti completed the largest dataset ever generated for a BL-4 agent by sequencing more than 100 full-length genomes from LASV directly from clinical samples.

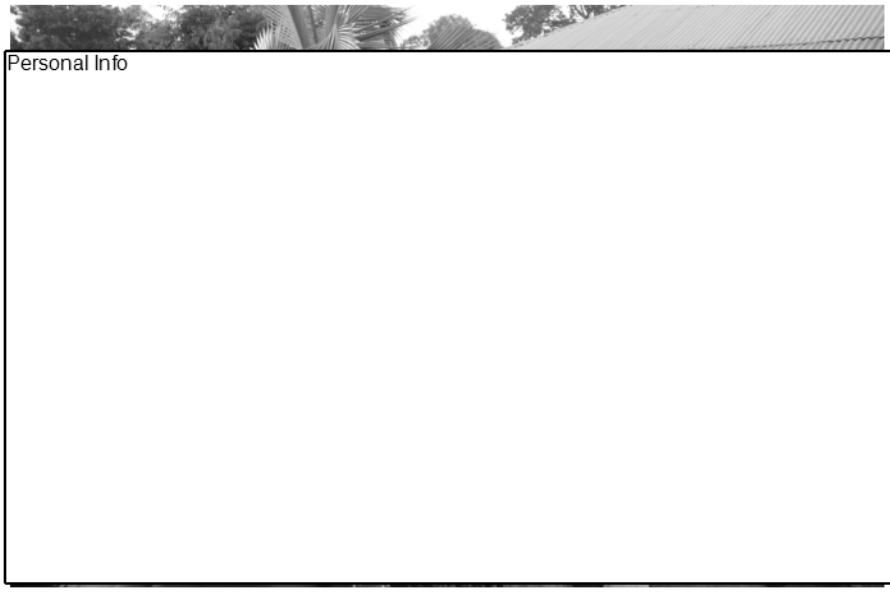


Figure 6. External Advisory Member Pardis Sabeti, Deputy Director of the Lassa fever program Dr. Donald S. Grant and Program Manager Simbirie Jalloh in a Mende village for Lassa Outreach (September 2013).

Erica Ollmann Saphire, PhD, Professor, The Scripps Research Institute (Fig. 7). The Ollmann Saphire lab determines the structural basis of viral pathogenesis, with a special emphasis on hemorrhagic fever viruses. She has determined all available structures of the oligomeric, prefusion filovirus GP in cleaved and uncleaved forms. We have also determined multiple crystal structure of arenavirus nucleoproteins, matrices, and glycoproteins. These structures have proposed new biological functions and structural rearrangements that could be targeted for antiviral design. In this effort, she have amassed hundreds of different constructs t o express the antigens, mapped multiple antibody epitopes, and have provided pioneering new insights into how the very few gene products of these viruses combine to cause pathogenesis. The driving force of Dr. Saphire's structural work is the opportunity to propose and develop new biological hypotheses about viral attachment, entry, and neutralization. Indeed, these structures have allowed her to propose the receptor-binding sites, consequences of cleavage, and conformational changes required for fusion with target cell endosomes and have opened multiple downstream lines of inquiry.

Specific Aim 8: To organize and oversee the analysis, presentation and publication of scientific data

The Administrative Core will coordinate preparation and internal review of manuscripts prior to submission for publication. Although the subject matter of most manuscripts will be plainly driven by the study protocol and design, Project/Core Leaders will be asked to submit informal manuscript "concepts" at an early stage so that the responsible investigator can be defined and authorship considered. Manuscripts will be prioritized for work by the Program Director and IAC. Once initial drafts of manuscripts have been completed, the Administrative Core will identify appropriate Project Program investigators (and external advisors as appropriate) to review them and provide constructive criticism.

Figure 7. External Advisory Group member Erica Ollmann Saphire at a nurses training session at Kenema Government Hospital.

Author order will generally follow the proportional contribution of the investigators for the work, regardless of seniority, with the "senior" or last author spot usually reserved for the important but

often more indirect contributions of the Program/Project/Core leadership. Recognizing that the results of any one Project or Core are, to some degree, dependent on the entire Project Program team, the policy regarding co-authorship will be to be as inclusive as legitimately possible, while still respecting the authorship guidelines of a given journal. When a large number of co-authors can legitimately be considered, a format of the primary authors' names followed by "for the Lassa ICIDR" will be used.

Specific Aim 9: To manage the sharing of data and resources with external investigators and the management of intellectual property

The data and unique resources generated through this Project Program will be made accessible to the general scientific community. The Administrative Core will manage requests for access to these various resources from verifiably qualified external investigators, with input from the IAC. Because LASV is a Select Agent, proper scrutiny of the requesting investigator and laboratory is warranted. In some cases, consultation with officials at NIH and CDC or other government authorities may be sought before responding to requests for data or resources. A resource- or data-sharing agreement may also be required. The final decision regarding outside collaborations and sharing of resources will be communicated to the external investigator by the Program Director or Project/Core Leader.

Specific Aim 10: To foster infrastructure development, training and education on detection, prevention, amelioration, and treatment of Lassa fever targeting both the scientific and general communities.

Figure 8. Lassa fever training in Koinadugu District on the 11th of February 2014 supported by Tulane/ Lassa fever project at KGH. Pictured is Dr. Donald S. Grant, co-director of Lassa ICIDR Project 1.

We believe that the KGH Lassa fever research program can with the proper training and commitment perform human clinical trials of Lassa fever diagnostics, therapeutics, immunotherapeutics and vaccines, now in advanced stages of development through NIAID supported research projects. After nearly forty years of research on Lassa fever, there is now great promise for controlling, treating or preventing this devastating disease, which is of major importance to Sierra Leone. Therefore, as an over-riding theme, the Lassa ICIDR program will promote the development of research capacity at KGH, with a particular emphasis on training Sierra Leonean research staff. The Core will sponsor a yearly symposium on Lassa fever, create a website, and support relevant training of laboratory and clinical staff and other investigators. Through the ongoing outreach activities the Lassa ICIDR team will also continue its sensitization and education campaigns in at-risk communities (Fig. 8). These programs will be expanded to meet external and internal educational and training objectives.

- The Lassa CIDR will propose a symposium on a relevant topic to be held at the annual meeting of the ASTMH.
- Short-term training of laboratory staff, postdoctoral fellows, or other investigators, including personnel from outside of the Lassa ICIDR, in the laboratory tools relevant to the study of immunology of Lassa fever and the VHFs. In most cases, these resources will be dedicated toward the training of colleagues from Sierra Leone.

- Training on working with Lassa virus. Many of the Lassa ICIDR personnel, especially Drs. Garry and Khan, have experience organizing and leading these types of workshops. Topics to be covered include:
    - Principles of good laboratory practices
    - Biocontainment and biosafety
    - Biosecurity
    - Principles of safe collection, transport, and storage of specimens
    - How to package and ship specimens
    - Dangerous goods shipping
    - Laboratory quality control
    - Data management and sharing
- A Lassa ICIDR website will be developed to provide scientific information on the Lassa ICIDR and the immunology of VHFs to relevant research communities. Links will be established to the website for the Viral Hemorrhagic Fever Consortium (vhfc.org) and other relevant websites of the various Project/Core investigators and laboratories, such as All the Virology on the World Wide Web (www.virology.net).

Please refer to the Projects, which have incorporated specific training programs related to development of clinical and laboratory research and data management (Project 1, Aim 3; Project 2, Aim 4), all geared to establish clinical trials capacity at KGH.

This is the first submission by Tulane University and the Lassa Fever Program at Kenema Government Hospital to the International Collaborations in Infectious Diseases Research (U19) program.

A Progress Report Publication List is not applicable.

The Administrative Core does not use Human Subjects. However, the Administrative Core will provide the investigators of Projects 1 and 2 regulatory and ethical compliance support for this aspect of their research. The Administrative Core will ensure that all Lassa ICIDR Human Subjects research is conducted according to the above policies, as well as all applicable laws and regulations. The Administrative Core will also provide training sessions in the ethical use of Human Subjects in research.

Human Subjects research conducted in Projects 1 and 2 will involve approximately equal numbers of male and female subjects. Since the subject enrollment will take place in West Africa, 100% of the study population is expected to be black African. There are no exclusions. Please refer to the Human Subjects sections of Projects 1 and 2 for further information about inclusion of Women and Minorities.

Children of all ages will be included in the Human Subjects research conducted in Projects 1 and 2. The research team includes healthcare workers and facilities with extensive experience in dealing with children. Please refer to the Human Subjects sections of Projects 1 and 2 for further information about inclusion of children.

The Administrative Core does not use vertebrate animals. However, the Administrative Core will provide the investigators of Project 1 regulatory and ethical compliance support for this aspect of their research. The Administrative Core will ensure that all Lassa ICIDR animal research is conducted according to applicable laws and regulations. The Administrative Core will also provide training sessions in the ethical use of animals in research.

Select Agent Research. The select agent to be used in the conduct of this research is Lassa virus. Both Tulane University and the Lassa Fever Program at Kenema Government Hospital (KGH) will comply with NIH guidelines on the handling of Select Agents. The Investigators will operate under all applicable Health and Human Services Department and Department of Agriculture regulations and Federal laws including the Patriot Act and the Tauzin Act. These requirements are designed to assure that potential bioterrorism agents and toxins are protected from loss and theft, and that personnel who work with them are trained, reliable and trustworthy. When appropriate, diagnostic specimens collected in West Africa will be inactivated, either through heat inactivation or placement in buffers (such as Tri-Reagent or Buffer RLT). NO viable LASV will be shipped under this contract.

Biosafety and Biocontainment. Biosafety and biocontainment policies and regulations are presently in place at KGH. These will be reviewed continuously by the Co-PI, PMs, and Internal and External Advisory Groups and reinforced where needed.

Biosecurity. A biosecurity protocol is in place for the Lassa Laboratory at KGH handling patient specimens which: 1. Describes inventory control procedures, 2. Contains provisions for routine cleaning, maintenance, and repairs, 3. Contains provisions for training personnel in safety and security procedures and for securing the area, 4. Describes procedures for loss or compromise of passwords and other possible security breaches, 5. Contains procedures for reporting suspicious persons or activities, loss or theft of specimens, or alteration of inventory records, 6. Contains provisions for the control of access to specimens, 7. Details safe operating procedures and procedures for reporting and removing unauthorized persons, 8. Contains provisions for ensuring that all individuals with access understand safety and security requirements and are trained and equipped to follow established safety and security procedures, 9. Establishes procedures for reporting and removing unauthorized persons, and 10. Establishes procedures for securing the area when approved individuals are not present.

Training on working with Select Agents. Because of the biosafety and biosecurity concerns of research on Select Agents, and the complicated regulatory environment, training sessions will be held at throughout the Lassa ICIDR Project at a minimum of a yearly interval. Many of the Project personnel, especially Drs. Khan and Garry, have experience organizing and leading these types of workshops, and the personnel at KGH are well-versed in biosecurity procedures. Topics to be covered include: Principles of good laboratory practices, Biocontainment and biosafety, Biosecurity, Principles of safe collection, transport, and storage of specimens, How to package and ship specimens, Dangerous goods shipping, Laboratory quality control and Data management and sharing

none

Tulane University, as the direct and primary recipient of the National Institutes of Health (NIH) grant funds, will be accountable to NIH for the performance of the project (Lassa ICIDR), the appropriate expenditure of grant funds by all parties, and all other obligations of the grantee, as specified in the NIH Grants Policy Statement on CONSORTIUM AGREEMENTS. In general, the requirements that apply to the grantee will also apply to the Lassa Fever Program of Kenema Government Hospital, the consortium participant.

Tulane University will enter into a formal written agreement with the Lassa Fever Program of Kenema Government Hospital that addresses the negotiated arrangements for meeting the scientific, administrative, financial, and reporting requirements of the grant, including those necessary to ensure compliance with all applicable Federal regulations and policies and facilitate a smoothly functioning collaborative venture.

The agreement will include:

- Identification of Robert F. Garry, John S. Schieffelin and Jeffrey G. Shaffer as the Co-Principal Investigators (Co-PI) with S. Humarr Khan (Co-PI), as the individual responsible for the research activity of the Lassa Fever Program of Kenema Government Hospital along with their roles and responsibilities;

- Procedures for directing and monitoring the research effort;

- Procedures to be followed in reimbursing the Lassa Fever Program of Kenema Government Hospital for its effort, including dollar ceiling, method and schedule of reimbursement, type of supporting documentation required, and procedures for review and approval of expenditures of grant funds at each organization;

- If different from those of Tulane University, a determination of policies to be followed by the Lassa Fever Program of Kenema Government Hospital in such areas as travel reimbursement and salaries and fringe benefits;

- Incorporation of applicable public policy requirements and provisions indicating the intent of the Lassa Fever Program of Kenema Government Hospital to comply, including submission of applicable assurances;

- A provision addressing ownership and disposition of data produced under the consortium agreement;

- A provision making the inventions and patent policy applicable to Tulane University and the Lassa Fever Program of Kenema Government Hospital and its employees in order to ensure that the rights of the parties to the consortium agreement are protected and that the grantee can fulfill its responsibilities to NIH;

- As appropriate, provisions regarding property (other than intellectual property), program income, publications, reporting, and audit necessary for Tulane University to fulfill its obligations to NIH.

- Tulane University is responsible for determining whether the Lassa Fever Program of Kenema Government Hospital has filed assurances with NIH that would cover its activities within the consortium and, if not, for ensuring that any required assurances or certifications are submitted to NIH.

- It Tulane University's responsibility to ensure that all sites engaged in research involving human subjects have an appropriate Office of Human Research Protection (OHRP)-approved assurance

and IRB approval of the research consistent with 45 CFR Part 46, and to comply with NIH prior approval requirements related to the addition of sites not included in the approved application.

- The grantee is responsible for obtaining NIH approval for any actions to be undertaken by consortium participants that require such prior approval.

- It is the responsibility of Tulane University to include applicable requirements of the policy statement in their written agreements and highlighted that agreements must also include a reference to the financial conflict of interest policy, intellectual property, and data sharing requirements. Lassa Fever Program of Kenema Government Hospital investigators are subject to the Financial Conflict of Interest policy of Tulane University for disclosing Significant Financial Interests that are directly related to Lassa Fever Program of Kenema Government Hospital's work for Tulane University. Cell lines, samples or other resources will be freely available to other investigators in the scientific community.

- Tulane University will ensure that the applicable government-wide cost principles and NIH cost policies are followed.

- The Consortium Agreement will include a policy on authorship and co-authorship on publications. Author order will generally follow the proportional contribution of the investigators for the work, regardless of seniority, with the "senior" or last author spot usually reserved for the important but often more indirect contributions of the Program/Project/Core leadership. Recognizing that the results of any one Project or Core are, to some degree, dependent on the entire Project Program team, the policy regarding co-authorship will be to be as inclusive as legitimately possible, while still respecting the authorship guidelines of a given journal. When a large number of co-authors can legitimately be considered, a format of the primary authors' names followed by "for the Lassa ICIDR" will be used.

- Tulane University will require consortium participants to comply with the requirements of OMB Circular A-133 or 45 CFR 74.26(d), as applicable, for audit of NIH grant funds expended by consortium participants.

School of Medicine

L. Lee Hamm, MD
Senior Vice President & Dean, School of Medicine
James R. Doty Distinguished Professor and Chair

Tulane University

February 9, 2014

Dr. S.H. Khan, M.B.C.h.B, MWACP
Physician Specialist In-Charge
Lassa Fever Program
Kenema Government Hospital
MOHS In-Charge, Int. Med. Department
Ministry of Health and Sanitation
Kenema, Sierra Leone

Dear Dr. Khan:

On behalf of the Tulane University School of Medicine, I am writing to provide my strongest support and endorsement of the work proposed in your grant application entitled, ""International Collaboration in Lassa fever Research (Lassa ICIDR)." We are fully supportive of this application for funding from the United Stated National Institutes of Health under RFA-AI-14-002 International Collaborations in Infectious Diseases Research (U19).

I have followed closely the work of your Co-Principal Investigator Dr. Robert Garry who has been working with you to develop modem diagnostic assays for Lassa fever, including rapid tests that can be employed at the bedside of patients. The facilities managed by you and your staff at Kenema Government Hospital have obviously been critical to this effort. Tulane University has enthusiastically supported your work in Lassa fever in the past, and shall continue to do so in this new and important initiative to further establish KGH as a center of excellence for Lass fever research and training.

In this regard, Tulane University has historically been amongst the leaders in international training programs, such as those outlined in your proposal. Having a skilled workforce will help ensure the sustainability of a viable Infectious Disease Research Center beyond the immediate period of the ICIDR initiative.

I also take this opportunity to note that Tulane University is committed to the dissemination of scientific data and technologies. We fully support the NIH data release program and data sharing network, which are essential to ensure that studies of under-represented African populations are utilized to their maximum.

As Dean of the School of Medicine at Tulane University I promise to work to overcome any administrative obstacles to support this important new international project.

All best wishes for success with your application.

Sincerely,

L. Lee Hamm, M.D.
Sr. Vice President & Dean, School of Medicine

Tulane Health Center
131 S. Robertson St., New Orleans, LA 70112, tel. 504 988-5462, fax 504 988-2945, www.som.tulane.edu
Mailing Address: 1430 Tulane Ave., #8001, New Orleans, LA 70112-2632

DEPARTMENT OF ORGANISMIC AND EVOLUTIONARY BIOLOGY
HARVARD UNIVERSITY

26 OXFORD STREET
CAMBRIDGE, MASSACHUSETTS 02138

February 12, 2014

Dr. Robert Garry
Department of Microbiology & Immunology
Tulane University Health Sciences Center
1430 Tulane Avenue, SL-38
New Orleans, LA 70112

Re: NIH ICIDR Letter of Support & Collaboration

Dear Bob,

I am writing with great pleasure to give my absolute highest support for your U19 application to the NIH's International Collaborations in Infectious Disease Research (ICIDR) program. With your impressive combination of experience and expertise, together with visionary scientific abilities and connections throughout West Africa, I believe you are perfectly suited to propose this program. I am continuously inspired by your scientific insight and commitment to improving health outcomes in resource-poor areas, and I sincerely hope to be a part of this wonderful proposal.

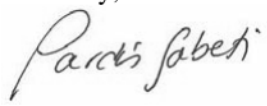
As you know, members of our Harvard team have been working since 2007 to establish a Lassa fever research program in Nigeria. My own program is an on-going effort modeled after your own Lassa fever research in Sierra Leone. We are currently conducting a genome-wide association study of Lassa fever in West Africa, and tied to that is an analysis of the evolutionary history of Lassa fever and an investigation of uncharacterized febrile illness in rural hospitals. With my own unique background in developing computational methods aimed at detecting signals of natural selection in the human genome, combined with your expertise on viral pathogenesis, immunology, and diagnostic development, we have had extraordinary successes studying this devastating and often overlooked disease. Having built impressive research networks throughout West Africa, we are now turning toward capacity building and scientific training for West African researchers to develop their own independent, fully functioning research programs. I believe this proposal capitalizes on this exciting time in West Africa, wherein many African universities and hospitals are adopting newer technologies, diagnostic tools, and research methods to bolster their own research agenda.

I believe you are uniquely suited to head this impressively designed program. Over the last decade, your work on Lassa fever diagnostics, in collaboration with African and US-based partners alike, have replaced assays based on Lassa virus grown in Biosafety level 4 laboratories in the United States, and they have positively impacted the treatment of Lassa fever in Sierra Leone. These new assays are more sensitive and specific than the BSL-4 assays, which were always in short supply, and, through your work, they have provided rapid LF diagnostics to villages of Sierra Leone that lack laboratory capacity and rarely have electrical power. More recently, I've watched you work closely with Nigeria's Ministry of Health to introduce recombinant antigen Lassa fever diagnostics in Nigeria, where the number of LF cases appears to be increasing dramatically due to annual, severe outbreaks. Your ability to maneuver seamlessly between New Orleans and Sierra Leone, working directly with hospital officials and local ethics committees while conducting compelling and impactful scientific research, makes you an ideal candidate to head an ICIDR program.

In recent years, our approach toward improving the ability to treat Lassa fever has been to develop the research capacity of the field sites in Sierra Leone and elsewhere in West Africa to an unprecedented degree. You have been instrumental in developing the plans that will replace the historic Lassa Fever Ward at KGH with a new, 48-bed Lassa Ward. The new Lassa Ward, which will be completely constructed by mid-2014, will provide the Government Hospital in Kenema with adequate capability for research on and and treatment of Lassa fever. It will also serve as a unique resource for testing new preventive strategies and antiviral therapeutics. Through this U19 program, we can make better use of this brand new space and have a tremendously positive effect on clinical care and infectious disease research throughout Sierra Leone.

In all aspects of our work together, I have felt fortunate and honored to be able to work alongside you. I give you my absolutely unwavering support in this program and look forward to many years of continued collaboration.

Sincerely,



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February 10, 2014

Dr. S.H. Khan, M.B.C.h.B, MWACP
Physician Specialist -Ocharge
Lassa Fever Program
Kenema Government Hospital
MOHS -Ocharge, Int. Med. Department
Ministry of Health and Sanitation
Kenema, Sierra Leone

Dear Dr. Khan:

I am writing to provide my strongest support for the work proposed in your grant application entitled, "International Collaboration in Lassa fever Research (Lassa ICIDR)" submitted for funding under RFA-AI-14-002 International Collaborations in Infectious Diseases Research (U19). I look forward to working closely with your team as a member of the Lassa ICIDR Advisory Board.

My laboratory is pursuing structure function analysis of all four arenavirus proteins, as well as antibodies against them identified in Lassa Fever survivors in Kenema. Our work will provide the necessary 3D templates by which we may interpret which antibody epitopes and which antiviral targets, in the glycoprotein, nucleoprotein, matrix protein, and polymerase are effective and why. This information will better help us design diagnostics and therapeutics for patient care.

The Lassa fever program at Kenema Government Hospital the only facility in the world that could potentially carry out human trials of a new Lassa fever drug or vaccine. Therefore, I am pleased to provide my strongest support for your grant application.

Best wishes in this new and important initiative to further establish KGH as a center of excellence for Lassa fever research and training.

Sincerely,

Erica Ollmann Saphire, Ph.D.

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CELEBRATING 20 YEARS OF SERVICE

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Dr. S.H. Khan, M.B.C.h.B, MWACP
Physician Specialist In-Charge
Lassa Fever Program
Kenema Government Hospital
MOHS In-Charge, Int. Med. Department
Ministry of Health and Sanitation
Kenema, Sierra Leone

February 28, 2014

Dear Dr. Khan:

This letter is to offer my enthusiastic support for your proposal entitled "International Collaboration in Lassa fever Research (Lassa ICIDR)", submitted for consideration under RFA-AI-14-002 International Collaborations in Infectious Diseases Research (U19). I will also be extremely honored to serve on the Advisory Board of the Lassa ICIDR.

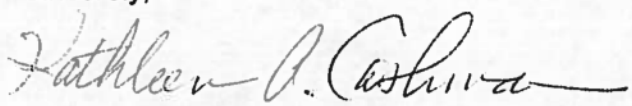
It is well known that ribavirin, though offered as current standard of care for Lassa fever when available, is only partially protective and is associated with significant side effects that limit its usefulness. Currently, there are at least two promising novel viral inhibitors with activity against Lassa fever virus. One of these, ST-193, a research product of SIGA Technologies, was screened in our laboratory *in vitro* against Lassa virus in cell culture, and was found to be efficacious against Lassa fever virus in a guinea pig model. This and other drug candidates, in order to establish their protectiveness in humans, will have to undergo stringent clinical evaluation. The Lassa fever program at Kenema Government Hospital is the ideal location for a clinical site, due to the experienced staff as well as the availability of a relevant patient population.

The focus of our current work is to continue the development of a DNA-based vaccine against Lassa fever virus that has been demonstrated to provide sterile immunity in guinea pig and nonhuman primate disease models against lethal infection. Among our endeavors is to understand the immune response to the vaccine in primates and distinguish a protective response from a non-protective response in this animal model such that we can develop an assay or set of assays that could reasonably predict a protective response in humans. As with furthering efforts to develop therapeutics, I cannot think of a better facility than the Kenema Government Hospital for human studies.

Our sincere hope is for the Lassa fever program at Kenema Government Hospital to maintain a successful facility, and to establish a strong collaboration with researchers in Sierra Leone to bring an efficacious vaccine to the people of West Africa. To this end, I am delighted to offer my full endorsement for the work proposed in this grant. It gives me great pleasure on a personal and a professional level to serve as a member of the Lassa ICIDR Advisory Board.

Those of us who engage in Lassa fever research do so in the hope that our work may ease some of the tremendous public health burden that this disease causes in endemic areas and beyond. I also hope that this grant will help foster additional collaborations that will benefit the Kenema Government Hospital and help advance the field of Lassa fever research as a whole.

Sincerely,



Kathleen A. Cashman, Ph.D.
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United States Army Medical Research
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Dr. S.H. Khan, M.B.C.h.B, MWACP
Physician Specialist In-Charge
Lassa Fever Program
Kenema Government Hospital
MOHS In-Charge, Int. Med. Department
Ministry of Health and Sanitation
Kenema, Sierra Leone

February 10, 2014

Dear Dr. Khan:

I am writing to offer my strongest support for the work proposed in your grant application entitled "International Collaboration in Lassa fever Research (Lassa ICIDR)", submitted for funding under RFA-AI-14-002 International Collaborations in Infectious Diseases Research (U19). It will also be my honor to serve on the Advisory Board of the Lassa ICIDR.

As you know, the only licensed antiviral agent with activity against Lassa fever, ribavirin, has had only mixed success and is associated with significant dose-limiting toxicities. Over the past years, novel classes of highly potent arenavirus inhibitors have been discovered and shown to protect against Lassa hemorrhagic fever infection in laboratory animal models. These drug candidates will ultimately require clinical evaluation. The Lassa fever program at Kenema Government Hospital is one of the few facilities, if not the only one, with the experience and patient population to carry out these clinical studies.

Among these promising new drug candidates, the nucleic acid base analog T-705 (favipiravir) has completed clinical evaluation in Japan and is currently undergoing Phase III studies in the US for the treatment of influenza. In collaboration with its developer, Toyama Chemical Co., my colleagues and I have shown that favipiravir is active against many important RNA viruses and, specifically, protects small animals against arenavirus infection. Importantly, and consistent with its excellent safety profile in humans, favipiravir does not inhibit the cellular enzymes thought to be responsible for ribavirin's dose-limiting toxicities. Based on preclinical efficacy and its advanced clinical development, favipiravir stands to be the first new Lassa antiviral that would benefit from the enhanced capabilities at KGH.

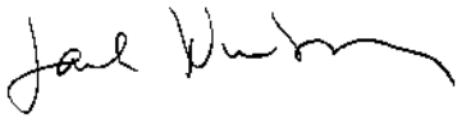
Another novel class of potential drug candidates comprises the small-molecule arenavirus entry inhibitors discovered by SIGA Technologies and workers at the Scripps Research Institute. Some of these compounds are specific to the South American hemorrhagic fever arenaviruses, some are specific to Lassa virus and some, such as ST-193, are broadly active against both groups. My laboratory has shown that these chemically distinct compounds bind a common site on the GPC envelope glycoprotein and act to antagonize its fusogenic activation by acidic pH in the endosome, thereby preventing virus entry and infection. Genetic, biochemical and virological studies indicate that these compounds bind to the pH-sensing interface between the fusion and stable-signal-peptide (SSP) subunits of GPC. ST-193 is orally bioavailable and has also been

shown to protect guinea pigs against lethal Lassa virus infection. Research is needed to further examine the breadth of antiviral activity in the endemic region, the pattern of antiviral resistance and the molecular basis for arenavirus entry and its inhibition. We hope to pursue these studies in collaboration with Sierra Leone researchers and pave the way for clinical development of a suitable drug candidate at KGH.

Therefore, I am pleased to provide my strongest endorsement for the collaboration proposed in your grant. I would be honored to serve as a member of the Lassa ICIDR Advisory Board.

Best wishes in this new and important initiative to further establish KGH as a center of excellence for Lassa fever research and training.

Sincerely,



Jack Nunberg, Ph.D.

February 10, 2014

Dr. S.H. Khan, M.B.C.h.B, MWACP
Physician Specialist In-Charge
Lassa Fever Program, Kenema Government Hospital
MOHS In-Charge, Int. Med. Department
Ministry of Health and Sanitation
Kenema, Sierra Leone

Dear Dr. Khan:

I am writing to provide my strong endorsement of your grant application entitled "International Collaboration in Lassa fever Research (Lassa ICIDR)", submitted for funding under RFA-AI-14-002. I will also be pleased and honored to serve on the Advisory Board of the Lassa ICIDR.

As you know there is as yet no approved Lassa fever (LF) vaccine. Ribavirin, the only available therapeutic, is most effective only during a short window early in infection, and the drug has severe adverse effects. Even with ribavirin treatment, fatality rates remain high, as our recently published studies have outlined. Passive transfer of antibodies is used as a treatment for Junin virus, the causative agent of Argentine hemorrhagic fever. Passive antibody transfer has also been shown to have efficacy in animal models of LF and in limited human studies. Passively delivered antibody is the only available medical product that may provide immediate specific immunity against a biological agent. Unlike vaccines, which require time to induce protective immunity and depend on the host's ability to mount a robust immune response, passive antibody can confer protection regardless of the immune status of the host.

There are currently over 30 FDA-approved monoclonal antibody based products, but Synagis (Palivizumab) is still the only approved antiviral antibody therapy (respiratory syncytial virus, RSV). As a key member of the team that developed Synagis, I and my research team at Zalgen are hoping to develop human monoclonal antibodies as therapy for LF. In collaboration with Tulane University and The University of Texas Medical Branch we have achieved 100% protection from lethal challenge with LASV in a newly developed guinea pig model after a single injection of certain neutralizing huMAbs. We also protected three of four Cynomolgous macaques from lethal challenge by injection of a single LASV huMAb. The huMAbs used in these studies are not our most potent huMAbs identified to date, and additional animal challenge studies will be conducted in the near future. We have also developed new methods for large scale production of huMAbs in serum-free GMP compliant cell lines, using a patented expression system, which will permit generation of therapeutic antibodies at greatly reduced cost. Fully human antibody therapeutics are the most advanced form of immunotherapeutics currently in development, highlighting the advanced technologies and approaches being employed by our consortium in the development and implementation of new countermeasures against LF.

Our consortium is hopeful that the unique resources of the LF program at Kenema Government Hospital (KGH) will enable us to test the efficacy of these promising new immunotherapeutics for LF in the near future. Therefore, I am pleased to provide my strongest support for your program to establish KGH as a center of excellence for Lassa fever research and training.

Very sincerely,

Luis M Branco, Ph.D., Founder, Zalgen Labs, LLC

Zalgen Labs, LLC
Germantown Innovation Center
20271 Goldenrod Lane, Suite 2083, Germantown, MD 20876
Personal Info mobile transfer; (301)515-5554 office; www.zalgenlabs.com

GOVERNMENT OF SIERRA LEONE
MINISTRY OF HEALTH AND SANITATION
OFFICE OF THE CHIEF MEDICAL OFFICER

Dr. S.H. Khan, M.B.Ch.B, MWACP,
Physician Specialist,
In-Charge, Lassa Fever Program
Kenema Government Hospital,
In-Charge, Internal Medicine Department
Kenema Government Hospital

24th February 2014

Dear Dr. Khan,

REQUEST FOR SUPPORT TO THE IMPLEMENTATION OF THE PROGRAMME ENTITLE "INTERNATIOAL COLLABORATION IN LASSA FEVER RESEARCH (LASSA ICIDR)"

The Ministry of Health and Sanitation has no objection for the collaboration between the Kenema Government Hospital and Lassa Fever Research (Lassa ICIDR). The MOHS is fully supportive of this application for funding from the United States National Institutes of Health under RFA-AI-14-002 International Collaborations in Infectious Diseases Research (U19). We are pleased that you will be serving as Co-Principal Investigator of this application.

The Government of Sierra Leone is committed to expanding access to knowledge on the part of its people and worldwide. It supports the development of Sierra Leone's capacity for internationally-renowned health research output. It aims to promote outstanding health research training and productive strategic relationships with partners in academia and private health research, as well as industry, commerce and government, in Sierra Leone and overseas. This in turn will accelerate national development aims, as set out in the Second Poverty Reduction Strategy.

The MoHS views development of a project in Sierra Leone in partnership with Kenema Government Hospital, Tulane University and the United States National Institute of Health as an important opportunity. The MoHS is also pleased to offer its firm commitment to participate as a member of the ICIDR network and is fully committed to indefinitely sustaining this important international research and training effort.

Best wishes for success in this important project that will greatly benefit the people of Sierra Leone.

Sincerely,

Dr. Brima Kargbo (GOOR)
Chief Medical Officer

MINISTRY OF HEALTH AND SANITATION, 4th Floor Youyi Building, Brookfields, Freetown
Contact Mobile No: Personal Info ***Email:*** Personal Info

GOVERNMENT OF SIERRA LEONE
MINISTRY OF HEALTH
KENEMA GOVERNMENT HOSPITAL

14th February 2014

Dr. S.H. Khan, M.B.C.h.B, MWACP,
Physician Specialist In-Charge
Lassa Fever Program
Kenema Government Hospital (KGH),
MOHS In-Charge, Int. Med. Department KGH
Min. of Health and Sanitation (MOHS)
Sierra Leone

Dear Dr. Khan:

Kenema Government Hospital and Laboratory hereby indicates its commitment to overcoming any administrative obstacles to the implementation of the"International Collaboration in Lassa fever Research (Lassa ICIDR)."

Our institutional commitment to the program includes the provision of adequate staff, facilities, and resources that can contribute to the planned program. There are numerous expert research staff, including yourself as Lassa ICIDR Co-Pi and Dr. Donald S. Grant as Co-director of Project 2, and Augustine Goba, Director of the KGH Laboratory that will ensure adequate training of new personnel. KGH also commits the facilities, space and resources necessary to conduct the day to day operations of Sierra Lassa ICIDR.

Kenema Government Hospital and Laboratory is also very pleased to offer its firm commitment to participate as a member of the ICIDR Network and to sustain the project after the funding period of the grant. There are a number of mechanisms that we will use for sustainability including the conversion to a fee for service facility and on going partnering with research partners that require unique research capacity.

Please do not hesitate to inquire if you require additional information.

Very sincerely,

.....
Dr. Christian Partt
Medical Superintendent (KGH)

14/2/014

Tulane University recognizes the necessity to balance the protection of intellectual property rights with the need to broadly disseminate new discoveries. Tulane University will follow the Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources (64 FR 72090) for: 1. Minimizing administrative impediments to the exchange of biomedical research tools; 2. Ensuring academic freedom and timely disclosure of research findings; 3. Ensuring appropriate implementation of the Bayh-Dole Act; and 4. Ensuring dissemination of research resources developed with NIH funds. Tulane University pledges to coordinate its efforts with those of the Lassa Fever Program at Kenema Government Hospital under such a contract to: i) protect intellectual property arising in the performance of the Lassa ICIDR grant; ii) facilitate the development for commercialization of the resulting therapeutic product; and iii) resolve disputes among the collaborating parties should such disputes arise in the performance of the contract. Specifically in these regards, Tulane University will ensure that intellectual property resulting from the Lassa ICIDR program is protected and developed in full compliance with the NIH guidelines and principles.

Tulane University will ensure that research materials developed during the work activities of the Lassa ICIDR program will be shared by all parties involved on terms essentially identical to those preferred by the NIH. Tulane University will work to further develop and commercialize resulting products, and will serve as the primary mediator among collaborating parties and will provide, to the best of its abilities, a neutral forum for resolution of any disputes. Tulane University shall be solely responsible for the timely acquisition of all appropriate proprietary rights, including intellectual property rights, and all materials needed to perform the project. Before, during, and subsequent to the award, the U.S. Government is not required to obtain for Tulane University any proprietary rights, including intellectual property rights, or any materials needed by to perform the project. Tulane University is required to report to the U.S. Government all inventions made in the performance of the project, as specified at FAR 52.227-11 (Bayh-Dole Act). Tulane University will work collaboratively after award with the Lassa Fever Program at Kenema Government Hospital to prepare a joint dissemination plan.

Research materials derived in the course of this work will be made accessible to the general scientific community will be provided to verifiably legitimate users for non-commercial uses without restriction.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0537858120000

Legal Name*: TULANE UNIVERSITY
Department:
Division:
Street1*: TULANE UNIVERSITY
Street2: 6823 ST. CHARLES AVE
City*: NEW ORLEANS
County:
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 701180000

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
Ms.	Kathleen	M	Kozar	

Position/Title: Director
Street1*: 1430 Tulane Avenue, Ep-15
Street2:
City*: New Orleans
County: Orleans
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 701122613
Phone Number*: 5049885613 Fax Number: 5049881748 Email: elecnot@tulane.edu

7. TYPE OF APPLICANT*

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Project 1: Evaluation of second generation Lassa fever immunoassays as point-of-care diagnostics and surveillance tools for Lassa fever.

12. PROPOSED PROJECT

Start Date*	Ending Date*
01/01/2015	12/31/2019

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	TULANE UNIVERSITY
Duns Number:	0537858120000
Street1*:	TULANE UNIVERSITY
Street2:	6823 ST. CHARLES AVE
City*:	NEW ORLEANS
County:	
State*:	LA: Louisiana
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	701180000
Project/Performance Site Congressional District*:	LA-002

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Kenema Government Hospital
DUNS Number:	8505106210000
Street1*:	1 Combema Road
Street2:	
City*:	Kenema
County:	
State*:	
Province:	
Country*:	SLE: SIERRA LEONE
Zip / Postal Code*:	
Project/Performance Site Congressional District*:	

File Name

Additional Location(s)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ○ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ● No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ● **Yes** ○ **No**

6.a. If yes, identify countries: Sierra Leone

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_1_Specific_Aims_Abstract.pdf
8. Project Narrative*	Pjt_1_Narrative_1.pdf
9. Bibliography & References Cited	Project_1_references.pdf
10.Facilities & Other Resources	Facilities__ICIDR_.pdf
11.Equipment	Lassa_ICIDR_Equipment.pdf

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

International Collaboration in Infectious Disease Research on Lassa fever (Lassa ICIDR)

Project 1: Evaluation of 2nd generation recombinant assays as point-of-care diagnostics and surveillance tools for Lassa fever.

Much of what is known about Lassa fever was elucidated in specialized isolation units in endemic areas and represents the most severe clinical manifestations of disease. Studies have shown that treatment with ribavirin is effective at controlling viremia in acute Lassa fever patients but is most effective when administered early in disease progression. However, onset of Lassa fever is gradual and nonspecific, making the disease difficult to recognize. Patients presenting with early signs of Lassa fever are nearly impossible to distinguish from other febrile diseases, such as malaria and typhoid, based on clinical findings alone. Patients are more likely to visit a peripheral health unit (PHU) during the early stages of disease, when clinical signs and symptoms of Lassa fever are nonspecific. These PHUs have limited laboratory capacity and personnel. Febrile patients are commonly administered antimalarial and antibiotic drugs and sent home, only to return days later with more severe disease. It can be several days after onset of symptoms and after no response from antibiotics that clinicians consider Lassa fever at which point patients are referred to the specialized units that can diagnose and treat Lassa fever patients. Over 75% of patients presenting to the Kenema Government Hospital (KGH) Lassa Ward had symptoms for over 7 days. The delay in treatment likely attributes to the 69% case fatality rate in LASV antigenemic cases seen at the Lassa Ward.

We hypothesize that building diagnostic capacity for Lassa fever at PHUs will enable community health workers to diagnose and refer suspected Lassa fever cases earlier, and as a result, are more likely to consider and screen for Lassa fever when febrile patients present to their PHUs. This will enable us to learn more about the early stages of disease. Specific Aim 1 is to evaluate deployment of next-generation Lassa fever recombinant antigen immunodiagnostics for point-of-care detection in peripheral health units. Specific Aim 2 is to determine feasibility of using reLASV diagnostics as an epidemiological tool for country-wide seroprevalence studies. Specific Aim 3 is to utilize recombinant LASV diagnostics to define correlates of immunity to Lassa fever in patient contacts with little or no disease thereby identifying resistance patterns and susceptible cohorts for Lassa fever. Specific Aim 4 is to develop the data collection and data management capacity at the Kenema Government Hospital (KGH).

International Collaboration in Infectious Disease Research on Lassa fever (Lassa ICIDR)

Project 1: Evaluation of 2nd generation recombinant assays as point-of-care diagnostics and surveillance tools for Lassa fever.

Through the proposed research we will determine whether building diagnostic capacity for Lassa fever at PHUs will enable community health workers to diagnose and refer suspected Lassa fever cases earlier, and as a result, are more likely to consider and screen for Lassa fever when febrile patients present to their PHUs. This will enable us to learn more about the early stages of Lassa fever.

Clinical and Laboratory resources in Kenema, Sierra Leone (ICIDR Projects 1 and 2)

Kenema Government Hospital (KGH) in Sierra Leone, West Africa is a 350-bed facility situated in the heart of the region with the world's highest incidence of Lassa fever. Because of the importance of Lassa fever as a bioterrorism and public health threat, KGH has developed an advanced clinical and laboratory research capacity. To our knowledge, this is one of two facilities continuously operated and dedicated to the care of patients infected with a Category A Select Agent anywhere in the world. The KGH Laboratory has the capacity and quality control to perform diagnostic testing for Lassa fever and is the only facility with laboratory testing and clinical treatment of Lassa fever in the entire country. In addition, the *Central Public Health Laboratory* (CPHL) in Liberia is accustomed to receiving samples from suspected cases of Lassa fever, safely packaging them (specific training on this matter has been conducted), and delivering them by ground transport to Kenema for diagnostic testing. Almost every other week, samples are sent from Liberia to Kenema.

Fig A. Sample Processing in the Kenema Government Hospital Lassa Laboratory. Samples are manipulated in class II biosafety cabinets by personnel wearing full personnel protective materials.

The enhanced physical and organizational infrastructure presents an unparalleled opportunity to access and test clinical samples in areas where Lassa fever is a frequent occurrence. The necessary administrative infrastructure for research on Lassa fever in Sierra Leone is also well established. Sierra Leone has an ethics committee to review and approve research protocols and Federal Wide Assurances from the U.S. Department of Health and Human Services. Ultimately, the high incidence of Lassa fever, along with the unique infrastructure and resources of our established public health and research network in West Africa offer unique opportunities to conduct detailed and integrated studies on a VHF and Category A Select Agent.

The Lassa Laboratory at KGH.

The KGH Lassa Laboratory is located on the grounds of the hospital, but in a stand-alone building constructed in 2005. Samples are manipulated in class II biosafety cabinets by extensively trained personnel wearing full personal protective materials (gowns, gloves, eye protection, mask and rubber boots) (Fig. A). The laboratory is comprised of an approximately 5,500 square foot building divided into a general clinical laboratory for routine diagnostics that services the entire hospital and a 700 square-foot specialized suite for manipulation of samples from suspected cases of LF (Fig. B). The building is equipped with redundant power sources, including municipal power (which is extremely sporadic), a solar power system (panels, batteries, controllers and inverters, and 100, 16 and 6 kilovolt generators. ELISA (antigen and IgM and IgG antibody), and lateral flow rapid test for Lassa fever are performed here. Access to the building and the BSL-3 suite is restricted. Negative airflow is maintained. The laboratory also possesses equipment and trained personnel for RT-PCR and cell culture (with separate PCR and cell culture suites – Fig. C). The cell culture suites are used to prepare sera and PBMCs for shipment back to Tulane for further

Fig. B. The Kenema Government Hospital Lassa Laboratory. The solar panels provide essential power for refrigerators, freezers and other essential instruments.

analysis and isolation of human monoclonal antibodies to Lassa virus. Established biosafety and biosecurity guidelines are maintained, with oversight by the Sierra Leone Ministry of Health and Sanitation, WHO and Tulane. The KGH Lassa Laboratory has undergone site review by NIH Program Officers and found to be acceptable. Mr. Augustine Goba, the KGH Lassa Laboratory Director, has been actively involved in Lassa fever research since the 1980's. Mr Goba supervises three technicians.

Fig. C. The structure shown, which is adjacent to the KGH laboratory, is constructed from prefabricated office containers donated by the United Nations, which have been subsequently refitted to serve as PCR clean rooms and cell culture suites. A. 2006 Toyota Land Cruiser (shown) is available to partly support the proposed project, but will soon require replacement.

The Lassa Ward at KGH

KGH current maintains a year-round 25-bed ward for the care of patients with LF at which up to 600 suspected cases seen yearly. The ward is staffed with a full-time team of doctors, nurses, and cleaners. The staff has extensive training and experience treating and caring for LF patients and the majority have over a decade of experience with LF patients. Standard guidelines for the isolation and management of patients with viral hemorrhagic fevers (VHFs) are maintained (65). All personnel entering patient care areas are required to wear appropriate personal protective equipment (PPE): surgical scrubs and gown, rubber apron, double gloves, rubber boots, mask and face shield. Personnel must wash their hands with a bleach solution after patient contact. The Lassa Ward has two dedicated ambulances for patient transport. All drivers are trained in the use of PPE. The patient care area of the ambulance and ward as well as all durable equipment are washed with a bleached solution after use by trained cleaners.

The KGH team has broken ground on a new 48-bed Lassa Ward that will replace this historic, but timeworn facility (Fig. D). Funding for the new Lassa Ward was provided by from the Naval Facilities Engineering Command (NAVFAC), heading the medical diplomacy missions of DoD, with technical support from Tulane and the Viral Hemorrhagic Fever Consortium (www.VHFC.org).

Kenema Government Hospital Lassa Fever Outreach Team

The outreach team at KGH is a highly experienced group of community outreach workers (Figs E and F). The team is heavily integrated into Sierra Leone's highly structured health surveillance system, working in close partnership with district medical and surveillance officers. The outreach team conducts LF case investigations country-wide which also includes contact tracing and updating local stakeholders. The team also is heavily involved in community education for the prevention and early detection of LF including implementation of rodent control interventions and sanitation marketing. The team is well-versed in survey methods including sampling strategies, questionnaire administration, and collection of blood and other specimens in community settings. and his knowledge of the communities in eastern Sierra Leone is extensive. The team currently consists of two junior and one experienced member in addtion to their supervisor. If project is funded, one additional staff will be added. Team members speak the majority of dialects found in Sierra Leone including Krio, Mende, Temne, and Kono. The outreach team supervisor, Lansana Kanneh has been with the LF outreach team since 1994 and was appointed supervisor in 2013. Mr. Kanneh is a vibrant public speaker. He is currently working on a diplomma in public health with support from Tulane University.

Fig. D. The Lassa Ward. The current Lassa Ward is a 25-bed facility with a full-time clinical and nursing staff dedicated to the treatment of Lassa fever patients (top left). This facility will be replaced with a much-needed 48-bed facility (funded by NAVFAC) with construction by Fajaha International Construction expected to be completed by Q3 2014. The new ward will contain offices, a pharmacy, medical records room as well as high and low containment wards. The rooms were specifically designed to deal with infection control issues associated with VHFs. Layout of the facility and example of current progress (middle panels) and details of the construction plan (lower panel) were developed with technical assistance from Jason Moses (Jason Moses Projects, NYC) and the VHFC.

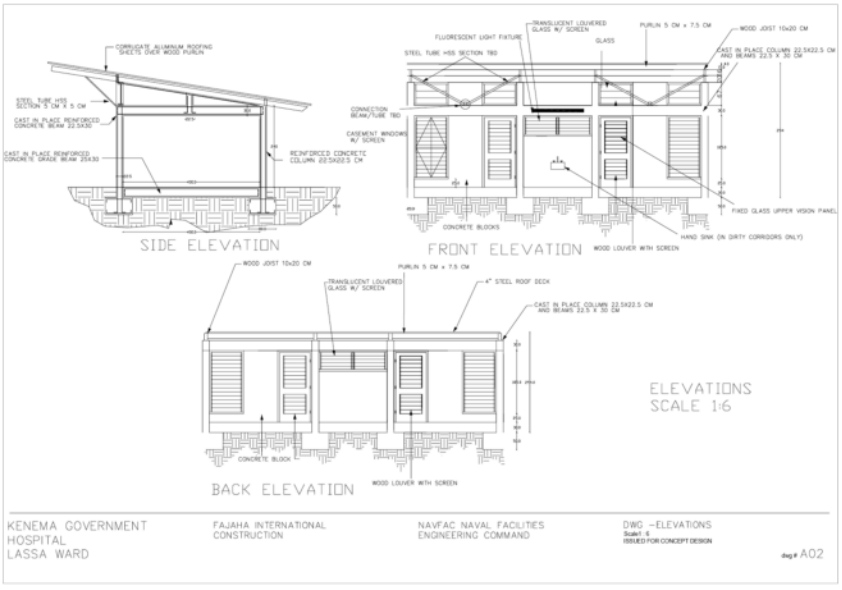


Fig. E. Community meetings with the KGH LF Outreach Team are done in evenings when subsistence farmers are back from their fields. . In addition to case investigations, the team also traps and distributes rodent control products to case households to prevent additional infections.

Fig. F. Mr. Lansana Kanneh, outreach team supervisor, performing informed consent and blood draws. Screening of normal or afebrile population clusters and identification of sub-clinically infected subjects in Project 1.

Kenema Government Hospital Lassa Fever EcologyTeam

The KGH ecology team has extensive knowledge on trapping of small mammals for zoonotic disease research (Figs. G and H). The team has expertise in field survey methods, live-capture trapping and sample preservation and storage. They also are experience rodent control and hygiene experts and provide a valuable resource for the development of LF prevention programs.

Fig. G. James Koninga (l) Kandeh Kargbo (m) and Lina Moses (r) during the necropsy of a juvenile *Mastomys natalensis*. In addition to biological specimens for virological testing, the team collects morphometric data for ecology focused research.

Fig. H. James Koninga (r) sets a Sherman live-capture trap in a corn field targeting *Mastomys natalensis.* Willie Robert (l) records position on transect line and documents GPS location for spatial studies.

Supervisor, Mr, James Koninga, has participated in collection of small mammals for zoonotic disease research since 1979 when he was trained by Centers for Disease Control and Prevention personnel during the first documented community epidemic of Lassa fever. Mr. Koninga has since participated in animal surveys during Ebola outbreaks and has received voucher preparation training from the University of New Mexico. Mr. Koninga is an expert in the safe handling and collection of biological materials from wild animals and ensures a high level of biosafety and sample quality assurance while in the field. Mr. Koninga supervises two junior ecologists for Tulane's research projects.

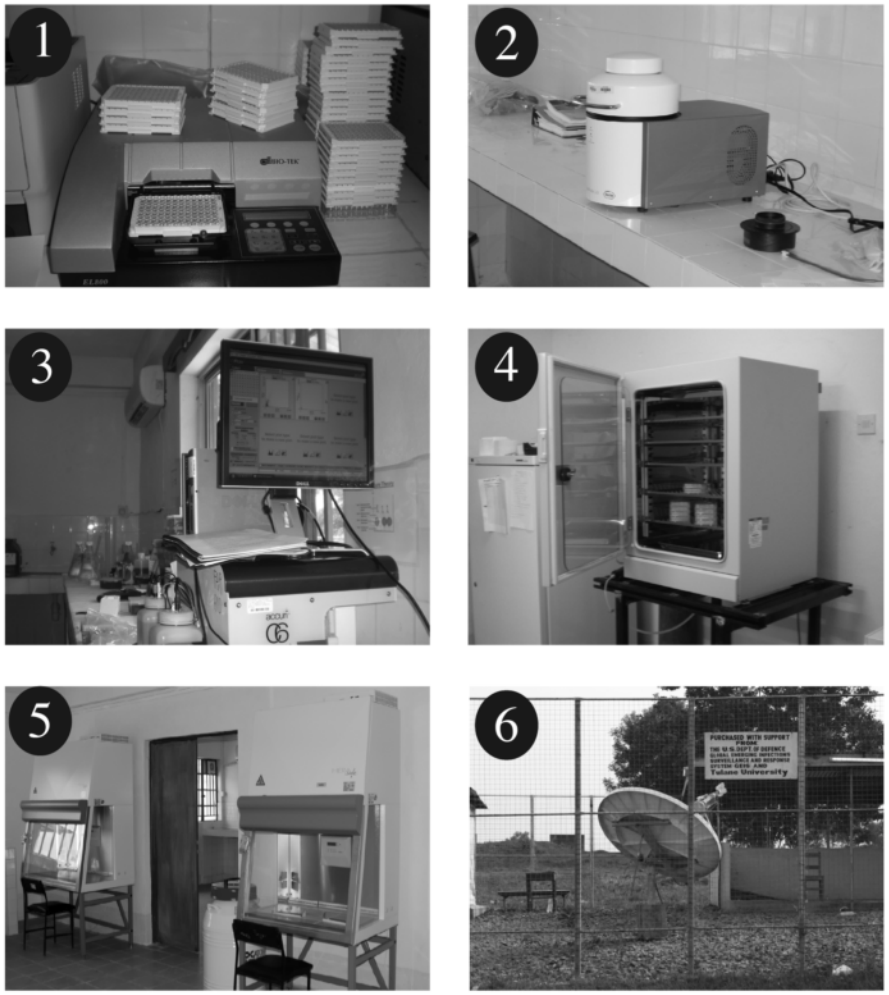


Fig α. Roche LightCycler 2.0 – real-time PCR machine in the KGH laboratory.

Kenema Government Hospital, Sierra Leone

Pertinent equipment items in the KGH Lassa Laboratory are shown in Figure α, including ELISA plate readers and washers (1), light-cycler real-time PCR machine (2), Accuri C6 flow cytometer (3), CO_2 incubator (4) class II biosafety cabinets (5), satellite internet communication system (6). The Lassa Laboratory also has LN2 tanks, a 4 liter per day LN2 generator, ELISpot reader, fluorescent microscope, conventional microscopes, table top centrifuges, microfuges, water baths, instrument sterilizer, solar and electric powered freezers, refrigerators, freezers, Millipore Direct Q water purification system, pH meter, vortex, balances and vacuum pumps.

Irrua Specialist Teaching Hospital, Irrua, Nigeria.

The laboratory facility is equipped with equipment to process and store blood samples and specimens, as well as RT- PCR and ELISA diagnosis of Lassa fever. These equipment include: 1 PCT-200 Thermocycler, 1 Sigma 415K Tabletop cold centrifuge, 1 Eppendorf cold microcentrifuge tube, 1 gel documentation system, Electrophoresis apparatus, 1 Barcoder and Barcoder stand, Micropipettes, Water distiller, 1 hood, 3 freezers, 1 fridge, 1 desktop computer, 1 autoclave and 1 digital stirred water bath.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Jeffrey Middle Name George Last Name*: Shaffer Suffix:

Position/Title*: Assistant Professor
Organization Name*: Tulane University
Department: Biostatistics
Division:
Street1*: 1440 Tulane Avenue
Street2:
City*: New Orleans
County:
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 701120000

Phone Number*: 5044328658 Fax Number: E-Mail*: jshaffer@tulane.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Co-PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 2008

File Name

Attach Biographical Sketch*:

Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Lina Middle Name Michiko Last Name*: Moses Suffix:

Position/Title*: Research Scientist
Organization Name*: Tulane University
Department: Microbiology and Immunology
Division:
Street1*: 1430 Tulane Avenue
Street2:
City*: New Orleans
County:
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 701120000

Phone Number*: 504-309-3233 Fax Number: E-Mail*: lmoses2@tulane.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Co-PD/PI Other Project Role Category:

Degree Type: BS,MSPH,PhD Degree Year: 2000, 2005, 2012

File Name

Attach Biographical Sketch*: Moses_Biosketch_final.pdf

Attach Current & Pending Support:

Funding Opportunity Number: RFA-AI-14-002 . Received Date: 2014-03-07T14:26:44.000-05:00

PROFILE - Senior/Key Person

Prefix: First Name*: Donald Middle Name Samuel Last Name*: Grant Suffix:

Position/Title*: Associate Director
Organization Name*: Kenema Government Hospital
Department: Lassa Fever Program
Division:
Street1*: 1 Combema Road
Street2:
City*: Kenema
County:
State*:
Province:
Country*: SLE: SIERRA LEONE
Zip / Postal Code*:

Phone Number*: 232 78 350 065 Fax Number: E-Mail*: Personal Info

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Co-PD/PI Other Project Role Category:

Degree Type: MbChB Degree Year: 2008

	File Name
Attach Biographical Sketch*:	biosketch_of_D_S__Grant_final.pdf
Attach Current & Pending Support:	

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Jeffrey	George	Shaffer		Co-PD/PI	Institutional Base Salary	EFFORT			14,630.00	3,760.00	18,390.00
2.	Dr.	Lina	M	Moses	Ph.D	Co-PD					24,385.00	6,267.00	30,652.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **49,042.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**49,042.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0537858120000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
1. Toyota Land Cruiser	45,727.00
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**45,727.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	25,000.00
Total Travel Cost	**25,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		3,000.00
2. Stipends		0.00
3. Travel		5,000.00
4. Subsistence		0.00
5. Other:		
2 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**8,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	35,736.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	76,495.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**112,231.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**240,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	117,778.00	59,478.00
		Total Indirect Costs	**59,478.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**299,478.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Proj_1_Budget_justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Jeffrey	George	Shaffer		Co-PD/PI	Institutional Base Salary	EFFORT			14,630.00	3,760.00	18,390.00
2.	Dr.	Lina	M	Moses	Ph.D	Co-PD					24,385.00	6,267.00	30,652.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **49,042.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**49,042.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0537858120000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
1. Motorcycle	10,000.00
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**10,000.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	25,000.00
Total Travel Cost	**25,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		3,000.00
2. Stipends		0.00
3. Travel		5,000.00
4. Subsistence		0.00
5. Other:		
2 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**8,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	50,863.00
2. Publication Costs	2,600.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	94,495.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**147,958.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**240,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	135,505.00	68,430.00
		Total Indirect Costs	**68,430.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**308,430.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Proj_1_Budget_justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Jeffrey	George	Shaffer		Co-PD/PI	Institutional Base Salary	EFFORT			14,630.00	3,760.00	18,390.00
2.	Dr.	Lina	M	Moses	Ph.D	Co-PD					24,385.00	6,267.00	30,652.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **49,042.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**49,042.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0537858120000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	25,000.00
Total Travel Cost	**25,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		4,000.00
2. Stipends		0.00
3. Travel		6,000.00
4. Subsistence		0.00
5. Other:		
3 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**10,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	58,863.00
2. Publication Costs	2,600.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	94,495.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**155,958.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**240,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	145,505.00	73,480.00
		Total Indirect Costs	**73,480.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**313,480.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Proj_1_Budget_justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Jeffrey	George	Shaffer		Co-PD/PI	Institutional Base Salary	EFFORT			14,630.00	3,760.00	18,390.00
2.	Dr.	Lina	M	Moses	Ph.D	Co-PD					24,385.00	6,267.00	30,652.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **49,042.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**49,042.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	25,000.00
Total Travel Cost	**25,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		4,000.00
2. Stipends		0.00
3. Travel		6,000.00
4. Subsistence		0.00
5. Other:		
3 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**10,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0537858120000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	58,863.00
2. Publication Costs	2,600.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	94,495.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**155,958.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**240,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	145,505.00	73,480.00
		Total Indirect Costs	**73,480.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**313,480.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Proj_1_Budget_justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT11602615 Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Jeffrey	George	Shaffer		Co-PD/PI	Institutional Base Salary	EFFORT			14,630.00	3,760.00	18,390.00
2.	Dr.	Lina	M	Moses	Ph.D	Co-PD					24,385.00	6,267.00	30,652.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **49,042.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**49,042.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
1. motorcycle	10,000.00
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**10,000.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	25,000.00
Total Travel Cost	**25,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		3,000.00
2. Stipends		0.00
3. Travel		5,000.00
4. Subsistence		0.00
5. Other:		
2 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**8,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	50,863.00
2. Publication Costs	2,600.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	94,495.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**147,958.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**240,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	135,505.00	68,430.00
		Total Indirect Costs	**68,430.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**308,430.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Proj_1_Budget_justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Senior/Key Personnel:
Jeffrey G. Shaffer, PhD, Co-Investigator, EFFORT is a Assistant Professor in the Department of Biostatistics and Bioinformatics at Tulane University School of Public Health and Tropical Medicine. Dr. Shaffer is an experienced statistician and data manager.

Lina M. Moses, PhD, EFFORT is Program Manager and Director of Ecology and Community-based research for Tulane's Lassa Fever Program in the Department of Microbiology and Immunology. Dr. Moses's expertise is in public health with specialties in community health, epidemiology and tropical medicine. She has worked on Lassa fever in Sierra Leone since 2007 and is Tulane point-of-contact for KGH Outreach and Ecology teams. Dr. Moses will oversee Aims 1-3 of Project 1.

Supplies:

	Year 1	Year 2	Year 3	Year 4	Year 5
Lassa ELISA reagents	27,000	27,000	27,000	27,000	22,000
Point-of-care metabolic panel tests	6,000	6,000	6,000	6,000	6,000
Lassa rapid tests	0	11,000	23,000	23,000	20,000
Research Lab reagents, PPE	2,736	2,863	2,863	2,863	2,863
Desktops and tablets	0	4,000	0	0	0
	$35,736	$50,863	$58,863	$58,863	$50,863

Lassa ELISA reagents are used for LASV screening and follow-up for all research subjects enrolled.

Point-of-care metabolic panel tests: $6,000 each year.

Lassa rapid test are used for distribution to peripheral health units for Project 1 Aim 1.

Research Lab reagents. laboratory consumables, sample storage, personal protective equipment.

Desktops and tablets $4,000 (Y2) are used for KGH staff bioinformatics capacity building—computer training and data collection, Project 1 Aim 4.

Equipment:
Two Honda XR motorcycles ($10,000 each) are requested for monitoring research activities in communities outside of Kenema Town. Costs in year 2 and 5 are for purchase of motorcycle, helmets, licensing, registration and insurance. Much of the community monitoring activities planned for the project can be performed by one individual, therefore transportation by motorbike ensures good value for money. Only experienced local personnel (non-Tulane staff) with motorcycle handler operator will operate motorcycles.

Funds are requested to purchase a Land Cruiser Hardtop, 6 Seater, Model: HZJ78L-RJMRS-05 in year 1 (For use in extreme climatic and physical conditions; $45,728, including delivery to Sierra Leone; quote via Toyota Gibralter), an essential piece of equipment to be used for fieldwork and sample acquisition in Sierra Leone.

Foreign Travel: $20,000 (Y1) + $25,000 (each Y2-5)
Airfare to and from Sierra Leone is approximately $2,500 for each round-trip. Excess baggage costs for transporting of supplies and equipment costs approximately $1500 per trip with an additional $500 for in-country travel. Travel for four trips for Dr. Moses or Dr. Shaffer is planned for each year of the project.

Training
Funds are budgeted in each year for tuition and fees and other costs related to Training Programs,

materials and travel costs for Sierra Leonean staff.

Indirect Costs:
The Indirect Cost Rate for the Tulane University School of Medicine (TUHSC) is 50.5% (Modified Total Direct). Indirect costs are not applied to equipment or Consortium costs.

Patient Care Costs: $55,000 (each Y1-5)
Funds are requested to cover outpatient costs of field staff monitoring Peripheral Health Units, collection of biological specimens in communities and follow-up of cases and controls. Also included is personal protective equipment and biological specimen collection. No funds will be used for investigational drug purchases.

Other expenses:
Vehicle fuel $11,000 (each Y1, 3-5) + $16,000
Funds will be used for fuel costs for field activities including oil and vehicle maintenance. The increase in Y2 is due to increased sampling in distant areas of Sierra Leone for Aim 2.2 of project 1.

Per diem $3,000 (each Y1-5)
To be used by key personnel during travel to Sierra Leone.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**245,210.00**
Section B, Other Personnel		**0.00**
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		**245,210.00**
Section C, Equipment		**65,727.00**
Section D, Travel		**125,000.00**
1. Domestic	0.00	
2. Foreign	125,000.00	
Section E, Participant/Trainee Support Costs		**44,000.00**
1. Tuition/Fees/Health Insurance	17,000.00	
2. Stipends	0.00	
3. Travel	27,000.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	12	
Section F, Other Direct Costs		**720,063.00**
1. Materials and Supplies	255,188.00	
2. Publication Costs	10,400.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	454,475.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		**1,200,000.00**
Section H, Indirect Costs		**343,298.00**
Section I, Total Direct and Indirect Costs (G + H)		**1,543,298.00**
Section J, Fee		**0.00**

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Donald	Samuel	Grant	M.D.	Co_PD	Institutional Base Salary	EFFORT			10,000.00	1,500.00	11,500.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **11,500.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	Nurses	EFFORT			9,000.00	1,350.00	10,350.00
3	Laboratory				9,000.00	1,350.00	10,350.00
3	Support staff				1,500.00	225.00	1,725.00
2	Drivers				4,000.00	600.00	4,600.00
4	Outreach/Ecology Teams				4,000.00	600.00	4,600.00
16	**Total Number Other Personnel**					**Total Other Personnel**	**31,625.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**43,125.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	10,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**10,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		3,000.00
2. Stipends		2,000.00
3. Travel		500.00
4. Subsistence		500.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**6,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	7,833.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. High speed Internet access	6,000.00
9. Software and computer supplies	3,537.00
Total Other Direct Costs	**17,370.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**76,495.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**76,495.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_1_KGH_Budget_Justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1. Dr.	Donald	Samuel	Grant	M.D.	Co_PD	Institutional Base Salary	EFFORT			10,000.00	1,500.00	11,500.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **11,500.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	Nurses	EFFORT			9,000.00	1,350.00	10,350.00
3	Laboratory				9,000.00	1,350.00	10,350.00
3	Support staff				1,500.00	225.00	1,725.00
2	Drivers				4,000.00	600.00	4,600.00
4	Outreach/Ecology Teams				4,000.00	600.00	4,600.00
16	**Total Number Other Personnel**					**Total Other Personnel**	**31,625.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**43,125.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	20,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**20,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		3,000.00
2. Stipends		2,000.00
3. Travel		500.00
4. Subsistence		500.00
5. Other:		
6 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**6,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,833.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. High Speed Internet access	6,000.00
9. Software and computer updates	3,537.00
Total Other Direct Costs	**25,370.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**94,495.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**94,495.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_1_KGH_Budget_Justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2017 **End Date*:** 12-31-2017 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Donald	Samuel	Grant	M.D.	Co_PD	Institutional Base Salary	EFFORT			10,000.00	1,500.00	11,500.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **11,500.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	Nurses	EFFORT			9,000.00	1,350.00	10,350.00
3	Laboratory				9,000.00	1,350.00	10,350.00
3	Support staff				1,500.00	225.00	1,725.00
2	Drivers				4,000.00	600.00	4,600.00
4	Outreach/Ecology Teams				4,000.00	600.00	4,600.00
16	**Total Number Other Personnel**					**Total Other Personnel**	**31,625.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**43,125.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2017 **End Date*:** 12-31-2017 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	20,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**20,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		3,000.00
2. Stipends		2,000.00
3. Travel		500.00
4. Subsistence		500.00
5. Other:		
6 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**6,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2017 **End Date*:** 12-31-2017 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,833.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. HIgh speed Internet Access	6,000.00
9. Software and Computer updates	3,537.00
Total Other Direct Costs	**25,370.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**94,495.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**94,495.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_1_KGH_Budget_Justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 4**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1. Dr.	Donald	Samuel	Grant	M.D.	Co_PD	Institutional Base Salary	EFFORT			10,000.00	1,500.00	11,500.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **11,500.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	Nurses	EFFORT			9,000.00	1,350.00	10,350.00
3	Laboratory				9,000.00	1,350.00	10,350.00
3	Support staff				1,500.00	225.00	1,725.00
2	Drivers				4,000.00	600.00	4,600.00
4	Outreach/Ecology Teams				4,000.00	600.00	4,600.00
16	**Total Number Other Personnel**					**Total Other Personnel**	**31,625.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**43,125.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	20,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**20,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		3,000.00
2. Stipends		2,000.00
3. Travel		500.00
4. Subsistence		500.00
5. Other:		
6 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**6,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,833.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. High Speed Internet Access	6,000.00
9. Computer and Software upgrades	3,537.00
Total Other Direct Costs	**25,370.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**94,495.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**94,495.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_1_KGH_Budget_Justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2019 **End Date*:** 12-31-2019 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Donald	Samuel	Grant	M.D.	Co_PD	Institutional Base Salary	EFFORT			10,000.00	1,500.00	11,500.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **11,500.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	Nurses	EFFORT			9,000.00	1,350.00	10,350.00
3	Laboratory				9,000.00	1,350.00	10,350.00
3	Support staff				1,500.00	225.00	1,725.00
2	Drivers				4,000.00	600.00	4,600.00
4	Outreach/Ecology Teams				4,000.00	600.00	4,600.00
16	**Total Number Other Personnel**					**Total Other Personnel**	**31,625.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**43,125.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2019 **End Date*:** 12-31-2019 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	20,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**20,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		3,000.00
2. Stipends		2,000.00
3. Travel		500.00
4. Subsistence		500.00
5. Other:		
6 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**6,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2019 **End Date*:** 12-31-2019 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,833.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. High Speed Internet Access	6,000.00
9. Software and Computer replacement	3,537.00
Total Other Direct Costs	**25,370.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**94,495.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**94,495.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_1_KGH_Budget_Justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Senior/Key Personnel:

Sheik Hummar Khan, M.B.Ch.B., Co-Director of Project 2 EFFORT is the Director of the National Lassa Fever Program of the Ministry of Health and Sanitation (MOHS) in Sierra Leone. He is permanently assigned to Kenema Government Hospital. He has over 10 years experience treating Lassa patients and recently completed a residency in Internal Medicine at Korle Bu Teaching Hospital in Ghana Dr. Khan is responsible for patient admission, testing and medical care on the Lassa Fever Ward. As Director of the National Lassa Fever Program, he also oversees activities of the Lassa Fever Laboratory and Outreach Teams.

Donald S. Grant, M.B.Ch.B, will serve as the Co-Director of Project 1 EFFORT and will be responsible for the overall supervision and coordination of all activities of the Project. Dr. Grant has extensive experience overseeing and managing research projects in Sierra Leone. Trained in Freetown, Sierra Leone Dr. Grant is Deputy Chief Clinician in the Lassa Ward, and is currently also a lecturer in the Community Health Department of the College of Medicine and Allied Health Sciences at the University of Sierra Leone. Along with his clinical work in the Lassa ward, Dr. Grant has been an investigator for a wide range of NIH-sponsored research studies. As an MOHS officer, his mandate is to build capacity, provide technical support, and coordinate regional efforts to confront LF and other communicable diseases in Sierra Leone, Liberia, and Guinea.

Laboratory:

Augustine J. Goba, Senior Laboratory Technician, Director of the Lassa Fever Laboratory. Mr. Goba has over 25 years of experience as a Senior Laboratory Technician having served as Chief Technician on the Centers for Disease Control and Prevention (CDC) Lassa Fever Project in Kenema, Sierra Leone from 1987 to 1994. During the Civil War in Sierra Leone, Mr. Goba worked as a Chief Laboratory Technician in the CDC Guinea Lassa Fever Project, N.'Zérékoré, Guinea before returning to Kenema after the War in 2005 to resume his position at the KGH Laboratory. Mr. Goba will be primarily responsible for performing the Lassa virus ELISAs and oversees routine hematology and chemistry testing of samples in the Lassa Fever Laboratory. He also supervises two medical technologists each with over five years experience, **Mohamed Fullah** and **Mambu Momoh**.

Nurses:

Mrs. Mbalu Fonnie, senior matron of nursing at KGH is a licensed nurse midwife and is the nursing supervisor of the KGH Lassa Fever Ward. She has over 30 years experience treating Lassa fever and specializes in the management of severe Lassa cases in pregnant women. Mrs. Fonnie began her career working at Nixon Methodist Hospital in Segbwema, Sierra Leone and participated in research trials conducted by the Centers for Disease Control. She supervises a nursing staff of nine nurses and nurse aids.

Ms. Veronica Koroma, assistant nursing supervisor and midwife, has over 15 years experience treating Lassa fever patients. She has devoted much of her life to the treatment and research of Lassa fever. Ms. Koroma is responsible for ensuring that nursing coverage is adequate and oversees the care of all acutely ill patients on a day-to-day basis.

Mr. Michael Gbakie is a registered nurse in Sierra Leone and serves as the Clinical Research Coordinator for the Tulane Lassa Fever Program. He has over 10 years experience with management and research of Lassa fever. Mr. Gbakie is responsible for the quality control of the onsite research. In this position, he is for ensures that all enrolled subjects are consented properly and that all case report forms are completed appropriately. In addition, he supervises a laboratory technician, **Sidiki Safa**, and works with the nursing staff to ensure that all laboratory testing is done per protocol. Mr. Safa also has over 20 years experience collecting blood samples from Lassa fever patients and has been a critical staff member for many years.

Support Staff:

Victor Lungay will serve Database Manager/Statistician and will oversee data entry, statistical analysis, and interpretation as he does currently for WHO-sponsored projects at KGH. Two part-time cleaners work in the Lassa Laboratory and Lassa Laboratory.

Drivers:

Drivers, mechanics (M Sow, M Fomgbeh EFFORT will support the Outreach and Ecology teams.

Outreach Team:
Lansana Kanneh a Sierra Leonean has 7 years experience in research projects with Tulane University and 20 years of experience on LF community education and surveillance. He is fluent in Mende and Krio and is extremely knowledgeable about the distribution of Lassa fever cases and geography of Sierra Leone. Mr. Kanneh, as supervisor of the Lassa Fever Outreach Team oversees case investigations and contact tracing of all Lassa fever cases diagnosed at IGH. In addition, he oversees and coordinates follow-up sample collections from Lassa patients discharged from the Lassa Ward. Mr. Kanneh will supervises a team of thee Outreach Team members.

Ecology Team:
Ecology Team leader Mr. James Koniga will supervise the ecology team and perform rodent trapping. Supervisor Koninga, has participated in collection of small mammals for zoonotic disease research since 1979 when he was trained by Centers for Disease Control and Prevention personnel during the first documented community epidemic of Lassa fever.Mr. Kargbo will be assisted by Kandeh Kargbo and Willie Robert.

Supplies:
Additional supplies and staff support are necessary for clinical research. Extra personnel are required for clinical research at this site including nurses, study coordinators, ambulance drivers and outreach workers for case investigation and patient follow-up visits. No funds will be used for routine clinical support or investigational drug purchases. Materials and supplies are needed for production of data collection forms, electronic tablets for data collection, GPS units, field team supplies.

Domestic Travel:
Aim II will require follow-up visits to locations outside of Kenema Government Hospital each year, requiring logistic support and fuel costs. Several of the regions are a day's drive from Kenema Government Hospital. Aim III requires multiple patient follow-up visits. Housing costs will be re-imbursed will be given to members of the Lassa Fever Program Outreach Team who must spend several days in the field for these visits.

Training:
Funds are budgeted in each year for tuition and fees and other costs related to Training Programs, materials and travel costs for Sierra Leonean staff that will be administered in Sierra Leone institutions, including the University of Sierra Leone (Freetown) and Eastern Polytechnic Institute (Kenema).

Other Expenses:
Town power in Kenema is highly unreliable. Town power is available about 5% of the time in Kenema, and even during those periods frequent intermittent or sustained power outages occur. Although the KGH Lassa Laboratory has limited solar power capability it is not sufficient to supply the power needs of the Lassa Ward, which relies on a generator. $8000 per year is requested for diesel fuel.

Funds are requested for maintaining the high-speed Internet connectivity amongst the Lassa ICIDR units ($6000 per year) and for upgrading software, computers and maintaining data management tools ($3,537 per year).

Indirect Costs:
None requested.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**57,500.00**
Section B, Other Personnel		**158,125.00**
Total Number Other Personnel	80	
Total Salary, Wages and Fringe Benefits (A+B)		**215,625.00**
Section C, Equipment		**0.00**
Section D, Travel		**90,000.00**
1. Domestic	90,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		**30,000.00**
1. Tuition/Fees/Health Insurance	15,000.00	
2. Stipends	10,000.00	
3. Travel	2,500.00	
4. Subsistence	2,500.00	
5. Other	0.00	
6. Number of Participants/Trainees	24	
Section F, Other Direct Costs		**118,850.00**
1. Materials and Supplies	71,165.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	30,000.00	
9. Other 2	17,685.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		**454,475.00**
Section H, Indirect Costs		**0.00**
Section I, Total Direct and Indirect Costs (G + H)		**454,475.00**
Section J, Fee		**0.00**

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
First Name*: Jeffrey
Middle Name: George
Last Name*: Shaffer
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ○ No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Project_1_Specific_Aims_One_Page.pdf
3. Research Strategy*	ICIDR_Proj1_jss_finala.pdf
4. Progress Report Publication List	Progress_Report_Publication_List.pdf
Human Subjects Sections	
5. Protection of Human Subjects	Human_subjects.pdf
6. Inclusion of Women and Minorities	Women.pdf
7. Inclusion of Children	Children.pdf
Other Research Plan Sections	
8. Vertebrate Animals	Vert_animals_ICIDR_Prjt_1.pdf
9. Select Agent Research	Admin_Core_manage_Select_Agent_Research.pdf
10. Multiple PD/PI Leadership Plan	Multiple_Pds_-_Project_1.pdf
11. Consortium/Contractual Arrangements	
12. Letters of Support	LoS_in_Admin_Core.pdf
13. Resource Sharing Plan(s)	Admin_Core_manage_Res_Sharing.pdf
Appendix (if applicable)	
14. Appendix	

Tracking Number: GRANT11602615 Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

International Collaboration in Infectious Disease Research on Lassa fever (Lassa ICIDR)

Project 1: Evaluation of 2nd generation recombinant assays as point-of-care diagnostics and surveillance tools for Lassa fever.

Much of what is known about Lassa fever was elucidated in specialized isolation units in endemic areas and represents the most severe clinical manifestations of disease. Studies have shown that treatment with ribavirin is effective at controlling viremia in acute Lassa fever patients but is most effective when administered early in disease progression. However, onset of Lassa fever is gradual and nonspecific, making the disease difficult to recognize. Patients presenting with early signs of Lassa fever are nearly impossible to distinguish from other febrile diseases, such as malaria and typhoid, based on clinical findings alone. Patients are more likely to visit a peripheral health unit (PHU) during the early stages of disease, when clinical signs and symptoms of Lassa fever are nonspecific. These PHUs have limited laboratory capacity and personnel. Febrile patients are commonly administered antimalarial and antibiotic drugs and sent home, only to return days later with more severe disease. It can be several days after onset of symptoms and after no response from antibiotics that clinicians consider Lassa fever at which point patients are referred to the specialized units that can diagnose and treat Lassa fever patients. Over 75% of patients presenting to the Kenema Government Hospital (KGH) Lassa Ward had symptoms for over 7 days. The delay in treatment likely attributes to the 69% case fatality rate in LASV antigenemic cases seen at the Lassa Ward.

We hypothesize that building diagnostic capacity for Lassa fever at PHUs will enable community health workers to diagnose and refer suspected Lassa fever cases earlier, and as a result, are more likely to consider and screen for Lassa fever when febrile patients present to their PHUs. This will enable us to learn more about the early stages of disease.

Specific Aim 1. Evaluation of the deployment of next-generation Lassa fever recombinant antigen immunodiagnostics for point-of-care detection in peripheral health units.

1.1: To assess impact of deployed rapid tests on time to referral from PHUs to KGH Lassa Ward.
1.2: To assess impact of deployed rapid tests on time to ribavirin treatment for LASV antigen positive patients.
1.3: To assess impact of deployed rapid tests on survival.
1.4: To develop guidelines on scale-up of rapid tests throughout Sierra Leone.

Specific Aim 2. Feasibility of using reLASV diagnostics as an epidemiological tool for country-wide seroprevalence studies.

2.1: To optimize and validate recombinant IgG assay using whole blood samples on filter paper.
2.2: To determine normal range of reactivity to recombinant LASV IgG in endemic, emerging and non-endemic regions of Sierra Leone.
2.3: To develop a strategy and build capacity for a country-wide LASV seroprevalence study.

Specific Aim 3. To utilize recombinant LASV diagnostics to define correlates of immunity to Lassa fever in patient contacts with little or no disease thereby identifying resistance patterns and susceptible cohorts for Lassa fever.

3.1: To determine incidence of LASV antigenemia in high-risk asymptomatic or preclinical LASV-infected contacts.
3.2: To contrast signs and symptoms of disease in LASV close contacts with LASV- close contacts and with LF cases presenting to KGH Lassa Ward.

Aim 4. Develop the data collection and data management capacity at the Kenema Government Hospital (KGH).

4.1: To improve the computer and data management competency among KGH staff.
4.2: To improve the process for collecting, managing, and storing data.
4.3: To link mobile phone use with access to patient care.

Research Strategy

Significance:

Much of what is known about Lassa fever (LF) was elucidated in specialized isolation units in endemic areas and represents the most severe clinical manifestations of disease (1-4). Studies have shown that treatment with ribavirin is effective at controlling viremia in acute LF patients but is most effective when administered early in disease progression (5-7). However, onset of Lassa fever is gradual and nonspecific, making the disease difficult to recognize. Patients presenting with early signs of LF are nearly impossible to distinguish from other febrile diseases, such as malaria and typhoid, based on clinical findings alone. Patients are more likely to visit a peripheral health unit (PHU) during the early stages of disease, when clinical signs and symptoms of LF are nonspecific. These PHUs have limited laboratory capacity and personnel. Febrile patients are commonly administered antimalarial and antibiotic drugs and sent home, only to return days later with more severe disease. It can be several days after onset of symptoms and after no response from antibiotics that clinicians consider LF at which point patients are referred to the specialized units that can diagnose and treat Lassa fever patients. Over 75% of patients presenting to the Kenema Government Hospital (KGH) Lassa Ward had symptoms for over 7 days (6). The delay in treatment likely attributes to the 69% case fatality rate in LASV antigenemic cases seen at the Lassa Ward.

Ruling out malaria by monitoring response to antimalarial treatment is part of the current World Health Organization case definition for a suspected LF case (8). This is problematic for many reasons. First, implicit in this method is an observational period after administration of anti-malarials of at least 24 hours which causes delay in care. In addition, antipyretics are often also administered, masking the signs of LF as patients begin to feel better. Lastly, one recent study revealed that 56% of laboratory-confirmed LF patients were also positive for malaria (RDT-confirmed), which is not surprising in the malaria hyper-endemic region of Sierra Leone. Screening patients for LF simultaneously with malaria will likely reduce delays for referral and treatment of LF.

Need for active community surveillance: Beyond the health care setting, few studies have characterized LASV transmission at community level where infection is most likely to occur. Much of what we know about LF epidemiology is from hospital-based studies (6, 7, 9-11). Lassa fever likely has a broad spectrum of severity, with hospitalized cases representing the most severe manifestations. Early prognostic markers for LF have yet to be characterized. In order to detect these early cases, active surveillance is needed. One approach would be to select defined populations and monitor for LF cases over time. This requires large personnel and resource inputs. Targeting high-risk groups, although not representative of overall risk, can yield more cases that surveillance in the general population. Although these groups are not representative of overall risk, they can help define such ciritical information such as infection to disease ratios and mild disease.

Recent Innovations in Diagnostics: We have reported on the expression, purification, and characterization of LASV proteins in bacterial and mammalian cell-based systems (12-16). Lineage IV LASV nucleoprotein (NP) has been successfully generated and purified as an MBP-fusion protein in *E. coli*, and a mammalian cell generated counterpart has been expressed in HEK-293T/17 cells in untagged and 6X-HIS-tagged formats. LASV glycoproteins have been expressed in mammalian systems as native GPC and uncoupled GP1 and GP2 subunits. Constructs expressing GP1 and GP2 were engineered using combinations of promoter elements, signal peptides, transmembrane (TM) and intracellular (IC) domains, and purification tags. The Z matrix protein has been expressed as a GST-fusion or in a 6X-HIS-tagged format. Panels of murine monoclonal antibodies (MAbs) and polyclonal antibodies have been generated against bacterial or mammalian expressed lineage IV LASV proteins. Over 100 distinct human monoclonal antibodies specific for LASV NP or GP have been isolated, cloned and characterized from LF survivors. Relevant hybridoma cell lines that produce IgGs specific to LASV NP, Z, GP1, and GP2 specificities have been adapted to growth in shaker and spinner flasks using serum free media formulations, for scale-up and streamlined purification of mAbs.

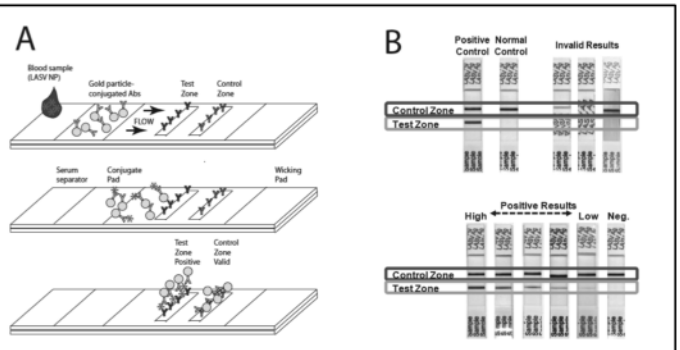


Fig. 1. Lassa fever lateral flow immunoassays. Panel A: schematic diagram of LF LFI (adapted from a Google image). Panel B. LF LFI interpretation from package insert.

Proof-of-concept for the use of recombinant LASV proteins in the development of a commercializable recombinant diagnostic platform for LF of multiple lineages, that includes ELISA and point-of-care lateral flow immunoassays (LFI), has been achieved with lineage IV assays. These recombinant assays are scalable and commercializable, two important features that will be advanced with the current project. Overall sensitivity (86.3%), specificity (97.9%), positive predictive value (86.3%), negative predictive value (97.9%) and diagnostic likelihood ratio (42.0) of the lineage IV LFI for acute LF meets or exceed comparable commercial RDT diagnostics for most other pathogens when used on patient samples obtained in Sierra Leone (17, 18).

The current LASV antigen detection LFI is formatted as a visually interpreted dipstick style rapid test (Fig. 1), meeting RFA requirements for ease of use, minimal operator training, random access, and cost-effectiveness. Other design features include a plasma separator sample receiving pad, a single test line and control line, and cover tapes for proper user orientation. The antibody detection LFI will incorporate these same features, but have separate test lines for IgM and IgG detection. Both immunochromatographic test strips will incorporate Ag or antibody specific control lines in the detection area to indicate valid results. Sample Buffers will be optimized for maximum analyte release into the test strip and assay sensitivity.

Innovation:
Studies proposed here will leverage an important advance, the development of recombinant LASV diagnostics that are sensitive and effective against LASV strains from lineage IV, present in Sierra Leone and surrounding countries. The infrastructure and resources of our public health and research network in Sierra Leone offers access to human subjects in the LASV endemic zones of West Africa, and the only opportunity in the world to extensively field-test diagnostic assays in real-time in patients with a severe viral hemorrhagic fever. Kenema Government Hospital (KGH) in eastern Sierra Leone is one of only two facilities in the world that house centers dedicated to LF treatment (Fig. 1, Resources). The cohorts of individuals with suspected LF in Sierra Leone that we assemble are novel. It is possible to access these patient groups for the first time in sufficient numbers because of the successes of our international research team, and the new facilities that have been developed at KGH. A new Lassa Ward at KGH, which is in the final stages of construction, offers further unique opportunities to conduct clinical aspects of our project. Access to human LF survivors provides a unique opportunity to utilize human IgG and IgM monoclonal antibodies (huMAbs) as calibrators and standards for immunoassays we will develop. The integration of Tulane University's research projects into MOHS activities uniquely positions this team to elucidate pre- and asymptomatic characterization of an endemic viral hemorrhagic fever.

This application focuses primarily on immunodiagnostics, rather than reverse transcriptase polymerase chain reaction (RT-PCR) or other nucleic acid diagnostics. We have observed that RT-PCR based assays perform poorly compared to immunoassays due in part to LASV sequence variation. While the facilities at KGH are advanced, they are highly prone to PCR contamination. An important reason to consider immunoassays over PCR methods is that the latter require instrumentation, expertise and facilities generally not available in the austere environments of the LASV endemic areas of West Africa. This application will also take advantage of the fact that ELISA can be converted to formats, such as lateral flow immunoassays that can be especially valuable for rapid diagnosis during an incident of bioterrorism and can also be used in the technology poor regions of West Africa. This is particularly important for health facilities in developing countries where LF is endemic and where the number of patients that fit the differential diagnosis of this disease can be considerable. Lateral flow immunoassays (LFI) are ideal for use as a point-of-care test in health care centers that lack the expertise and facilities to perform more complex assays, and could also greatly improve response times in the event of LASV deliberate release. LFI are a commonly recognized format for malaria test and tests for other pathogens in the West African subregion and are readily accepted.

The proposed work has the potential to change existing paradigms in LF diagnosis and treatment. The possibility of having laboratory tests done quickly and results available immediately is essential for successful intervention and management of LF. Early treatment with ribavirin and/or passive transfer of neutralizing antibodies can dramatically reduce mortality [19]. Availability of improved diagnostics will also empower significantly advances in the knowledge base of LASV epidemiology and natural history. More broadly, ongoing efforts by many investigators worldwide have resulted for the first time since LASV was first reported over forty years ago in the accumulation of sufficient scientific information to begin to achieve control of LF through the use of new therapeutics, including immunotherapeutics, and vaccines.

Approach

Specific Aim 1. Evaluation of the deployment of next-generation Lassa fever recombinant antigen immunodiagnostics for point-of-care detection in peripheral health units.

Rationale: Early initiation of ribavirin in LF patients is most likely to occur if they are diagnosed at a PHU during the early stages of LF. The *objective* of this aim is determine if distribution of point-of-care diagnostics will increase early referrals to KGH and decrease overall mortality secondary to LF. We will test the *hypothesis* that rapid tests in PHUs will increase referrals of suspected LF cases to the Lassa Ward and reduce the time to ribavirin treatment in confirmed cases. The rationale for this aim is that successful completion of the proposed research will demonstrate that point-of-care testing for VHFs can be safely and effectively deployed over large areas with limited resources. Such findings are of immense importance in the early detection and diagnosis of other infectious diseases throughout the tropics. **Innovation: Application of low-complexity point-of-care diagnostic test to tackle a health problem that has historically been hampered by challenges in production and scale up.** This is the first viral hemorrhagic fever point-of-care test to be used in resource-poor healthcare settings with limited laboratory capacity. Because of the high incidence of LF in Sierra Leone, we are in the unique position to be able to assess impact of this technology on healthcare outcomes.

Research Design:

Specific aim 1.1: To assess impact of deployed rapid tests on time to referral from PHUs to KGH Lassa Ward, we will **monitor peripheral health units in Kenema District for rate of referral of suspected LF cases before and after deployment of rapid tests.** Hypothesis: Rapid tests in PHUs will significantly increase the rate of referrals of suspected LF cases to the Lassa Ward.

Sampling population and design. Forty PHUs in two chiefdoms of Kenema District, Sierra Leone will be randomly selected and monitored for 12 months to determine rate of referral for suspected Lassa fever cases. Rate will be defined as number of suspected LF cases referred in the last month. After 12 months, PHUs will be randomly split into units that receive the rapid tests and units that do not receive the rapid tests. Health care workers at deployment PHUs will receive training on how to properly run the assay and education on surveillance of LF. Units not receiving the tests will receive only education on surveillance of LF. Units will be monitored for an additional 12 months to determine referral rate after deployment of tests. Twelve months is necessary to the study because of the seasonality of LF. A randomly selected control group followed simultaneously will allow for detection of any external factors that may influence study results, such as increased training and LF awareness among health workers.

Linear regression will be used to compare PHUs receiving the rapid tests and PHUs which do not. Outcome of interest will be referral rate. Rates of referral from the first year of project will be compared to rates after deployment. Rates will also be compared between PHUs not receiving the rapid test and those that do receive the tests.

Expected outcomes: We expect quick uptake and use of rapid tests in PHUs and an increase in referrals to the KGH Lassa Ward. This will have a positive impact on other aims in the study, as we expect an increase in LF cases.

Specific aim 1.2: To assess impact of deployed rapid tests on time to ribavirin treatment for LASV antigen positive patients, we will **follow individuals referred from PHUs with and without LASV rapid tests and document time (in days) from date of onset of illness to date of ribavirin administration and from date of referral to date of ribavirin administration.** Hypothesis: Rapid tests in PHUs will significantly reduce the time to ribavirin treatment for LF patients.

Sampling population and design. Referrals of suspected LF cases from the PHUs monitored in Aim 1.1 will be tracked upon referral to KGH Lassa Ward. Study personnel will interview referrals to determine approximate time at onset of symptoms. They will also track time from first clinic visit. After the first year of tracking, rapid tests will be deployed to the randomized PHUs and referrals will continue to be monitored for an additional year.

Linear regression will be used to determine if deployment of rapid tests are associated with reduced time to treatment. Patients presenting from PHUs with rapid tests will be compared to patients before these PHUs received rapid tests as well as PHUs not receiving rapid tests. Outcome of interest will be duration from onset to treatment in days.

Expected outcomes: We anticipate rapid tests at initial presentation will dramatically reduce the time to ribavirin treatment.

Specific aim 1.3: To assess impact of deployed rapid tests on survival, we will **follow individuals referred from PHUS with and without LASV rapid tests and document death or survival to discharge.** Hypothesis: Individuals referred from PHUs with rapid tests will have lower mortality than individuals referred from PHUs without rapid tests.

Sampling population and design. Suspected LF cases referred to the KGH Lassa Ward will be tracked from the PHUs recruited in Aim 1.1 and monitored for survival to discharge or death. As in Aim 1.2, after the first year of tracking, rapid tests will be deployed and survival will continue to be monitored in patients referred from PHUs.

Logistic regression will be used to determine survival/death among patients from PHUs receiving rapid tests compared to survival/death in patients at the same PHUs before they rapid tests are deployed. Individual referrals will also be compared to patients from PHUs which do not receive the tests. Outcome of interest will be survival/death.

Expected outcomes: We predict that deployment of rapid tests will result in patients receiving care earlier in the disease, thus reducing mortality.

Specific aim 1.4: To develop guidelines on scale-up of rapid tests throughout Sierra Leone, we will **engage key MOHS personnel and other key stakeholders to develop a strategy for distribution of rapid tests for LF.**

Based on the findings in Aims 1.1-1.3, we will be able to discern if deployment of rapid tests at the PHU level has a positive impact on patient outcomes. Through this aim, we will identify challenges with distribution and supply as well as cost-benefit. We will work with key personnel in the Sierra Leone Ministry of Health and Sanitation, particularly directorates of Hospitals and Laboratories and Disease Prevention and Control, to develop a national scale-up strategy for deployment of the rapid tests, be it at hospital or PHU level.

Expected outcomes: We will produce a national-level strategic plan for LF diagnostics at community level.

Specific Aim 2. Feasibility of using reLASV diagnostics as an epidemiological tool for country-wide seroprevalence studies.

Rationale: In Sierra Leone, LF has historically been found in the Eastern Province. In recent years, LF has appeared and is increasingly common in areas outside of the Eastern Province. Whether this is actual expansion of endemic areas or increased diagnostic capacity and awareness remains unclear. The *objective* of this aim is to determine if reLASV diagnostics can be used as an epidemiologic tool. Our *first hypothesis* is that large-scale blood spots collections can be reliably tested using recombinant ELISA-based platforms. The *rationale* for this aim is that accurate LASV seroprevalence rates are necessary to assess LF risk and burden in endemic countries. When the proposed study of Specific Aim #2 is complete, we will have a greater understanding of how to utilize innovative diagnostic platforms as epidemiologic tools.

An estimated 80% of LASV infections in humans are believed to be mild or asymptomatic (19). This estimate is based on seroprevalence studies done using convenience sampling of communities (20-22). These studies have revealed a high number of IgG positive individuals that do not recall a severe febrile disease. Unfortunately, these seroprevalence studies were not done using probability-based sampling, so it is unknown how distribution of IgG and LASV fluctuates across landscapes and specific demographic groups at population levels. A countrywide seroprevalence survey would provide insight into this shift in distribution of LASV. It would also provide valuable information to the country of Sierra Leone to develop evidence-based policy and health strategies. Further, understanding the distribution of LASV would provide needed background for future ecology and epidemiology research to better understand LASV transmission. Undertaking such a large-scale survey would require capacity building not just in laboratory scale-up, but in field sampling capacity.

Preliminary Studies: Case investigations are a routine part of the public health follow-up when a LF case is identified through the KGH Lassa Fever Laboratory. The KGH Outreach team interviews the LF case (if conscious) and family members at the hospital, then visits the case house for signs of rodent infestation,

performing rodent extermination on the site. They seek out any close contacts that may have symptoms consistent with Lassa fever and refer them to the Lassa Ward. In 2012, we began collecting samples on all close contacts for LASV Ag, IgM and IgG screening. We have had high participation using this sampling method, and believe that with increased resources, we will be able to recruit from this engaged population.

Table 1.1 Contacts enrolled

Year	Number enrolled	Ag+	IgM+	IgG+	Ag-/IgM-/IgG-
2012	173	2	28	72	60
2013	217	3	54	46	99

Recombinant IgG ELISA assay. We are currently developing standard cut-off values and titration curves for our IgG ELISA assay using samples collected from a previous study. From 2007-2008, our team undertook a population-based seroprevalence study across Kenema District in eastern Sierra Leone to determine the proportion of the population that had been previously exposed to LASV (Moses et al., manuscript in preparation). We recently examined 700+ samples collected in this study for LASV-specific IgG by recombinant ELISA (rELISA). Initial screening using antigen from LASV glycoprotein revealed 61% of individuals sampled had OD levels at least 2 standard deviations above normal controls. Screening using LASV nucleoprotein (NP) identified 68% of individuals sampled had OD levels at least two standard deviations about normal controls. Further validation of our findings is underway including titration of individual serum that were positive by initial screening (>2 s.d. above negative controls). These results indicate that the objective of Specific Aims #2 and #3, which require the participation of several communities and the collection and testing of several hundred samples, is feasible with our team. In addition, the recombinant IgG assay is performing well in healthy individuals with limited background that makes interpretation of results difficult.

Research Design:
Specific aim 2.1: To optimize and validate recombinant IgG assay using whole blood samples on filter paper, we will **store blood on filter paper under various environmental and cold storage conditions of varying length.** Hypothesis: antibodies from whole blood stored on protein saver filter paper will be stable at room temperature and give sufficient biological material to run the IgG assay in duplicate.

Sampling population and design. As part of the country-wide serosurvey, blood specimens will be obtained using a finger stick on filter paper (Whatman® 903 protein saver, GE Healthcare Life Sciences, Inc.). The use of a single finger stick rather than venepuncture will ensure higher participation and less time required to obtain biological sample. We will obtain blood by finger stick from a panel of LASV- (Ag, IgM, IgG) and LASV+ (Ag, IgM and or IgG) and place blood on filter paper. We will then store filter papers under various conditions including ambient temperatures and 4^{0}C for various time periods ranging from one week to six months. We will then develop a standard operating procedure to obtain blood sample and sample storage.

Expected outcomes: We anticipate antibodies on filter paper will remain stable in ambient temperatures for up to three months. This will enable us to perform large-scale surveys on LASV distribution in Sierra Leone.

Specific aim 2.2: To determine normal range of reactivity to recombinant LASV IgG in endemic, emerging and non-endemic regions of Sierra Leone, we will **collect whole blood on filter paper from 100 individuals in three LF zones (endemic, emerging, non-endemic) and contrast these samples with a panel of normal human serum from the United States.** Hypothesis: Normal range of negative subjects will not vary between LF zones, but IgG+ prevalence will vary significantly.

Sampling population and design. A convenience sample of self-identified healthy people from a) LF endemic areas, b) LF emerging areas, c) non-endemic areas. We will recruit 100 individuals in the three LF zones, collecting in multiple villages in each zone.

We will screen all samples along with a panel of commercial normal human serum (NHS) using the recombinant IgG ELISA. Sierra Leonean subjects with optical densities (OD) exceeding the 95^{th} percentile (3 standard deviations in normally distributed data) obtained from the NHS panel will be considered positive for previous exposure to LASV. Individuals exceeding the 90^{th} percentile (2 standard deviations) but below the 95^{th} percentile will be considered indeterminate. We will use linear regression modelling to determine if

the three endemic zones (endemic, emerging, non-endemic) are statistically different from each other. If no difference is detected, we will use general cut-off values for the entire country. If the regions do differ, we will utilize region specific cut-off values for each group.

Expected outcomes: We will have validated and standardized cut-off values for large-scale IgG ELISA testing.

Specific aim 2.3: To develop a strategy and build capacity for a country-wide LASV seroprevalence study, we will **collaborate with Statistics Sierra Leone to identify enumeration areas from the 2014 census for random sampling based on population proportional to size and mapping.**

Sampling population and design. We will engage Statistics Sierra Leone, the governmental office responsible for the national census to identify enumeration areas used in the 2014 census. We will randomly select 10 enumeration areas in each district to verify population size within each enumeration area. We will also map these enumeration areas using GIS mapping.

Expected outcomes: In collaboration with Statistics Sierra Leone and Sierra Leone MOHS, we will develop a survey manual for surveyors for a country-wide serosurvey.

Specific Aim 3. To utilize recombinant LASV diagnostics to define correlates of immunity to Lassa fever in patient contacts with little or no disease thereby identifying resistance patterns and susceptible cohorts for Lassa fever.

Rationale: Much of what is known of LF has been gleaned from hospital-based studies representing the most severe cases. Asymptomatic and mild LASV infection is not well-characterized. In order to detect these cases, active surveillance is needed. One approach would be to select specific populations and monitor for LF incidence. This requires monitoring for detection of new cases and involves a large investment of resources and personnel to detect few cases. The *objective* of this aim is to identify immunologic differences between symptomatic and asymptomatic LF cases. Our *working hypothesis* is that asymptomatic LF cases differ from symptomatic ones in terms of antigen levels and serologic markers. When this aim is complete, we will have identified early prognostic markers of LF illness course and outcome in individual patients.

Specific Aim 3.1: To determine incidence of LF antigenemia in high-risk asymptomatic or preclinical LASV-infected contacts, we will **collect blood sample and basic demographic information from all close contacts of laboratory-confirmed LF cases presenting to the Lassa Ward.** Hypothesis: Screening of close contacts will reveal a greater number of asymptomatic and mildly ill cases than severely ill LF cases.

Sampling population and design. Study personnel will conduct case investigations in the community where a LF case originated from. Personnel will also identify individuals who came into direct contact with the LF case. This will be our cohort to follow for Aims 3.1 – 3.3. The study will be explained, consent form is obtained, and subjects will be screened for LASV infection (Ag, IgM+, IgG). Individuals with positive IgM will be screened 4-7 days later to detect greater than 4 fold increase in IgM titre. These individuals, along with the LASV Ag+ patients will be considered Lassa fever cases and incidence will be calculated among these individuals.

Expected outcomes: We anticipate an large number of asymptomatic or mildly symptomatic LASV-infected contacts, relative to acutely ill contacts. This will enhance sample size and objectives of Specific Aim 3.2.

Specific Aim 3.2: To contrast signs and symptoms of disease in LASV+ close contacts with LASV- close contacts and with LF cases presenting to KGH Lassa Ward, we will **follow close contacts recruited in specific aim 3.1 and document LASV Ag, IgM, and IgG, and metabolic parameters over time**. Hypothesis: LASV+ close contacts will differ from comparison groups in antigen levels, and specific metabolic tests.

Sampling population and design. LASV+ contacts and LF cases will be followed daily for ten days. Interviews will be administered to determine at-risk behaviours and previous illness history. They will be rescreened for LF using the recombinant ELISAs as well as a metabolic panel (Na, K, TCO2, Cl, Glu, Ca, AlkPhos, ALT, AST, TBili, Alb, TP, Hgb) at time points described in Table 1.2.

Table 1.2 Contacts recruited in Aim 3.2 will be classified in the following groups:

Subject Classification	Classification Criteria	Data collection timepoints (Ag, IgM, IgG by ELISA, metabolic panel)
LASV+ contact	Contact that is Ag+ by ELISA or IgM+ with four-fold increase in IgM titre 4-7 days after initial sampling	Day 0, 1, 3, 5, 7, 10 Week 2 Month 3, 6
IgG- Contact	Individual negative for all three ELISAs (Ag, IgM, IgG)	Day 0 Week 2 Month 3, 6
IgG+ Contact	Individual negative for Ag and IgM by ELISA, but positive by IgG assay	Day 0 Week 2 Month 3, 6
LF case	Individual presenting to the KGH Lassa Ward who have Ag+ and/or IgM+ with ribavirin treatment course	Day 0, 1, 3, 5, 7, 10 Week 2 Month 3, 6

LASV+ contacts will be evaluated against all other comparison groups modelling LASV+ with the metabolic panel. Estimates of expected enrolment of this cohort are described in Table 1.3 based on recruitment rates from 2012 to 2013. We believe we will have sufficient sample size to detect a difference between LASV+ contacts and other comparison groups with an OR≥4 with power=0.8 and confidence level of 0.95.

Table 1.3. Estimated enrolment, Aim 3.

	Per year	4 years
Total LASV+ contacts	26	104
Ag+	5	20
IgM+ with increasing titer	21	84
Comparison groups		
IgG- Contact	125	500
IgG+ Contact	70	280
LF case	80	320

Expected outcomes: We expect contrasting and significantly different biomarkers in mild and asymptomatic LASV-infected controls. This will enable us to establish early biomarkers that will be predictive of patient outcome and inform better patient management.

Aim 4. Develop the data collection and data management capacity at the Kenema Government Hospital (KGH).

Specific aim 4.1: To **improve the computer and data management competency among KGH staff. Hypothesis:** Based on a before and after skills assessment, hospital personnel completing the computer course significantly improve their computer skills.

Several recent NIH-funded research projects supporting the joint efforts of Tulane University and the KGH have led to remarkable infrastructural developments at the KGH, including a stabilized electrical power system, stable internet service, and new computer equipment. These efforts have laid the groundwork for improving the computer competency of personnel at the Kenema Government Hospital (KGH). Currently, the KGH employs a paper-based data collection system where data are maintained using a suite of relational databases and spreadsheets. The data is backed up using a combination of external hard drives and cloud-based storage for some of the key study data. This process is somewhat inefficient and depends on the operational flow of paperwork, which often precludes hospital staff from accessing vital patient data in a timely manner. Further, handwritten entries are often difficult to decipher, which makes this two-stage process of data collection and data entry particularly prone to errors. Maintaining data on local computer hard drives commonly results in multiple data files that differ based on a trail of modifications or updates. The current process is also prone to delays in data availability due to the time lapse between data collection and data enty. The process requires paper forms to be completed and transported by hospital staff between the Lassa ward and laboratory buildings and thus provides a means for the transfer of hazardous biological material at a site where large-scale Lassa fever outbreaks have occurred (19).

The stable internet system at the KGH provides the opportunity for implementing web-based data entry and storage operations, which are widely considered to be more reliable than offline methods. The introduction of mobile data collection tools would likely be effective for carrying out data collection operations in remote areas in Sierra Leone due to their portability and would also provide a solution for addressing the inherent problems of a two-stage data collection and entry system. With an underdeveloped grid and little telephone line infrastructure, the widespread coverage and usage of cell phones across Africa (23) provides a communication link between patients and medical personnel. However, to our knowledge, optimizing their role in Lassa fever patient care has not previously been studied in Sierra Leone.

Significance: Provides capacity building by improving the computer skills for KGH staff which will directly expand the local capacity for collaborating in research projects. The structured and archived nature of the course materials will allow for sustained training.

Innovation: Seeks to alter the current "on demand" training practices with formal, structured training workshops. The workshops will be customized for the Kenema region and laid out in the context of Lassa fever.

Research approach 4.1: A three-part course sequence will be developed and delivered (on-site) to relevant KGH personnel. Prior to developing the courses, several focus group discussions will be held by Tulane personnel and key KGH personnel to determine the focus of the course topics. The feedback and conclusions drawn from these discussions will be used to develop preliminary course syllabi, which will then be submitted to Dr. Grant for his approval. Three courses will be developed in accordance with trainee computer literacy levels. The courses will structured in increasing level of difficulty (basic, medium, and advanced topics) and will emphasize competencies in computer fundamentals, data management practices, and data collection practices. Each course will be laid out as a series of presentations modules that will be delivered as a three-day, on-site workshop. Courses will be held in the KGH library, which has a maximum capacity of approximately 20 trainees. The mode of instruction will be interactive, where the first part of each course module will consist of a lecture, and the latter part will consist of hands-on computer exercises. A course manual will be developed to expand on concepts and will serve as a reference guide. Each course will conclude with a performance examination and certificates will be awarded upon successful completion of the attendance and exam requirements. After delivering the courses in a workshop format, they will be further developed into audiovisual training courses using the Tegrity system (24), where Dr. Shaffer will work with Tulane technology staff to develop each course into audiovisual Tegrity courses. These courses will be archived at the KGH site and used for subsequent trainees. Five personal computers and five tablet computers will be used to perform the training. Two trainees will be assigned to a single computer or tablet, which will allow a maximum of 10 subjects per training session. The **basic training course** will focus on competencies such as computer terminology, use, and maintenance; and applications such as the Microsoft Office suite. The **medium training course** will focus on primarily on data quality, assurance security, and sharing; and the **advanced training course** will focus on case studies for using computer tablets in field data collection.

To evaluate each course, a set of pre- and post-instruction exams will be developed. The pre- and post-instruction exam questions will be different but similar in level of difficulty and concepts. Each exam will consist of 50 multiple-choice questions and a single open-ended question. The exam scores will be compared using descriptive and inferential statistical techniques will be used to test the hypothesis that there is at least a 50% improvement following the training intervention. Each test will be complemented online evaluations using an online survey tool, and these results will be used to improve and develop the courses.

To promote the sustainability of the courses, trainees will be considered as "certified" to teach a course after successfully attending the course and scoring at least 90% of the questions correctly on its corresponding post-examination. All of the course materials and Tegrity modules will be made available to KGH staff for providing instruction to new trainees. Dr. Shaffer will be designated to develop and teach the courses and thereafter an on-site facilitator will be identified to continue the course offerings.

Specific aim 4.2: To **improve the process for collecting, managing, and storing data. Hypothesis:** Online data entry systems and mobile data collection methods result in more complete data, require less workload, and are preferred (by KGH personnel) over traditional data entry and collection methods.

Significance: Results in more efficient and accurate data collection methods and precludes data loss. The aforementioned techniques permit data audit trails and are likely a viable solution for issues related to data file conflicts and updates.

Innovation: Introduces a major proposed change to traditional data management and collection procedures at the KGH. These activities will determines whether online data management and mobile data collection methods are feasible at the Kenema site and specifically evaluates the proposed changes using hypothesis-driven substudies.

Research approach 4.2: The approach will execute a three-phased evaluation of electronic data capture and entry for hospital, laboratory and outreach operations. Each phase will be classified and carried out in terms of software development, on-site training, and evaluation against current practices.

Phase I: Hospital data collection. The Research Electronic Data Capture (RedCap;(25)) application will be used to build an online database and a suite of digital questionnaire forms that correspond to the questionnaires currently used for data collection. The digital forms and databases will be developed such that they do not maintain any personally identifiable information. The system will also allow for offline data entry and subsequent online synching in the event of power failure. Personal computers will be placed at the KGH Lassa Ward laboratory buildings. These computers will be linked via a local server and set up with active data connections. Current data collection personnel will be required to complete all of the webinars and tutorials at the RedCap resource site (26). This training will be complemented with on-site training in using specific RedCap features, including data entry, audit trails, source data verification, and security control. These personnel will then be responsible for logging incoming data into the RedCap online database. The paper-based system will be used throughout the study and will serve a backup and a basis for comparison. The online data will be maintained on a Tulane server, but the aforementioned local server will also be used to provide data access in the event of internet outage. A pilot study will be conducted to compare the online and offline data entry mechanisms. The study will include 50 consecutive consented subjects, where data collection and entry will be performed using the current method (data collected using paper forms and entered into an offline database) and the evaluation method (data collected using paper forms and entered into an online database). The mechanisms will be compared with respect to their (1) form-specific completeness; (2) observation-specific completeness; and (3) observable errors. We hypothesize that there is no difference in data loss between the current system and the RedCap approach.

Phase II: Laboratory data collection. A thorough review of the electronic laboratory notebook (ELN) applications will be reviewed and compared with respect to their cost, ease of use, workload, software compatibility, and their performance in settings with low bandwidth. Feedback will be solicited from laboratory personnel to assist in choosing an application. A system will be set in place to upload raw laboratory ELISA data from Ag, IgM, and IgG tests into the ELN. A local server will be used to provide a mechanism for file sharing and data backup between the KGH Lassa Ward and laboratory. A technique will be established to link the electronic laboratory data with the RedCap data. The system will be laid out and developed by Dr. Shaffer, and the KGH staff will be trained on their use. The current system of spreadsheets and macros will continue to operate in addition to the ELN-based data entry. The paperbound laboratory notebook will be continued and serve as the source document. Over three months, laboratory personnel will be trained to use both approaches. At the end of the three months, these approaches will be compared with respect to their time to availability. We hypothesize that the time to data production is faster using the ELN system than the current spreadsheet and macro system.

Phase III: Outreach data collection. Computer tablets will be reviewed according to their cost, durability, and functionality. A set of Windows-based computer tablets with protective cases will be selected and purchased for off-site evaluation. A suite of tablet-based digital questionnaire forms will be developed with conditional logic and validation functionality. The digital forms will be tested for synchronization with the RedCap system. All of the tablets will be encrypted to protect against theft or loss of device. A field manual will be developed and serve as a guide for all field data collection personnel. During an on-site visit, outreach personnel will be trained on tablet use and tablet-based data collection. The tablets will then be used to in conjunction with paper-based collection to provide a means for comparison and feasibility. Current data collection personnel will be responsible for carrying out data collection using both paper-based computer tablet approaches. These personnel will also be trained to upload the data to the RedCap online database. A pilot study will be carried out by comparing the completeness of key study data between the two approaches. Specifically, the results will be compared with respect to (1) form completeness; (2)

observation completeness; and (3) observable errors. We hypothesize that there is difference in key data loss between the paper-based and computer tablet data collection approaches.

Each phase will be tested to the fullest extent possible at the Tulane site prior to its implementation at the KGH. A representative from the KGH will be designated to serve as a leader and primary contact for each phase. Data collection personnel will be selected such that their current duties are simply replaced with digital entry duties to preclude any job losses. A monthly teleconference will be set up to monitor progress and garner local feedback and insight from KGH personnel. A biannual, on-site testing and training session will be used to monitor and improve the quality of each phase and study. Finally, a written recommendation will be derived from the results of each phase to initiate policy changes or justify the continuation of existing policies. We believe that, with very little infrastructure cost (save for the cost of the satellite internet system), that the proposed digital data management and collection system is sustainable and will return a reward after the cost of initial investment.

Specific Aim 4.3: To **link mobile phone use with access to patient care. Hypothesis:** Mobile phone use plays a role in presenting to the KGH.

Significance: Determines how focused interventions on cell phone use should be carried out to improve access to LF-related patient care. The lack of media infrastructure in Sierra Leone has long been a major impediment for communicating health information, but the rise of cell phone use across Africa has the potential to fill this gap. The relationship between cell phone use and patient care is not well understood across West Africa. Determining how cell phone usage relates to LF care will determine whether focused interventions for additional coverage and cell phone availability would reduce LF incidence, improve detection, and provide a starting point for exploring how they may be used in patient care. These findings also have the potential to improve communications between patients and doctors and facilitate follow-up visits, particularly for those in rural areas where coverage is sparse.

Innovation: Explores the potential of a rapidly growing technology for improving patient care. Efficient communication is particularly important for LF as treatment is most effective within 6 days following illness onset. Further, patient follow-up is rarely conducted due to lack of communication and resources. This aim attempts to shift the current self-reporting paradigm by increasing media communications between patients and doctors. Since literature on cell phone use in Sierra Leone is scarce, this effort will provide information on the current status of cell phone use and provide a platform for its use for competing illnesses.

Research approach 4.3: A sample of 200 consecutive incoming patients will be enrolled and surveyed regarding their cell phone coverage, ownership, and usage. Cell phone users will be defined as owning or having direct access to a cell phone. Although there is no preliminary data available on cell phone usage for the Kenema site, we estimate that at least 30% of subjects will be considered cell phone users, which will yield at least 60 subjects in each comparison group. Cell phone users will be excluded if they do not provide consent for receiving follow-up text messages. The comparisons of interest are: (1) cell phone users vs. non-cell phone users; (2) subgroup comparisons among cell phone users; and (3) subgroup comparisons among non-cell phone users. All patients will be asked about whether they own an operational cell phone and how it influenced their access to LF patient care. For cell phone users, survey questions will be used to assess:

1. Whether and how the phone was used in obtaining care (e.g., direct communication with referral center; call to village leader; call to family member);

2. The mode of communication (text messaging, voice, etc.);

3. Whether the cell phones are used to receive or communicate health information; and

4. The type and expense of mobile service.

Among non-cell phone users, the questions will be used to determine:

1. The reasons for not having a phone (coverage, cost, power); and

2. Whether indirect access to a cell phone (outside of the home) influenced their access to patient care.

For all subjects, data on will be collected on age, gender, occupation, district of residence, method or site of referral, urbanicity, clinical signs and symptoms, date of illness onset, LF test result, and survival outcome. These characteristics will be compared between the cell phone user and non-cell phone user groups. Results will be compared across methods and sites of referral to determine whether the results can be linked with referral center practices. These groups will also be compared to illness duration (at presentation) and survival outcome (at discharge). A wellness index will be generated using a set of self-reported symptoms to determine whether illness severity is related to cell phone use. Cell phone users will be asked to allow for a follow-up reminder via text message to determine post-discharge recovery. The ability to communicate with these subjects will be measured with direct telephone correspondence. For subjects that cannot be reached, attempted communication to next of kin or emergency contact will be used to determine ultimate survival outcome. These results will then be used to determine whether patients with cell phones that receive text messages more likely to comply with follow-up recommendations.

A cell phone coverage map will be generated to illustrate spatial trends in cell phone coverage and usage. A written recommendation will be developed on whether or how cell phones can be used to address the needs of the most severely ill patients and implement patient care in a timelier manner.

Project 1 Concept Proposal: Evaluation of 2nd generation recombinant assays as point-of-care diagnostics and surveillance tools for Lassa fever.

	Aim 1	Aim 3
Title	Evaluation of the deployment of next-generation Lassa fever recombinant antigen immunodiagnostics for point-of-care detection in peripheral health units.	To utilize ReLASV diagnostics to define correlates of immunity to Lassa fever in patient contacts with little or no disease thereby identifying resistance patterns and susceptible cohorts for Lassa fever.
Hypothesis	Deployment of point-of-care rapid diagnostics to peripheral health units (PHUs) will increase referrals to the LF Ward and reduce time to ribavirin treatment and mortality.	Active LASV+ case surveillance through contact tracing will identify early symptomatic and asymptomatic LASV-infected individuals. These individuals will have different biomarkers than those presenting at KGH through clinic referral and those without active LASV infection.
Objectives	Deploy LASV rapid kits to 40 PHUs in Kenema District	Identify a cohort of early and asymptomatic individuals with evidence of active LASV infection and contrast them to non-LASV and late presenting LF cases
Clinical Sites	Lassa Fever Ward, Kenema Government Hospital 80 MOHS-sponsored PHUs in Kenema District	Lassa Fever Ward, Kenema Government Hospital
Eligibility Criteria	1.1: Any MOHS-supported Community Health Center or Maternal-Child Health Center willing to be monitored. 1.2 and 1.3: LF case confirmed by Ag+ ELISA or IgM+ with clinical signs and symptoms referred from one of 80 PHUs identified in Aim 1.1.	LF case: individual presenting to the KGH LF Ward with signs and symptoms of LF and Ag+ or IgM+ LASV+ contact: individual with history of physical contact with LF case during illness and LASV Ag+ or IgM+ (with 4-fold increase in titre at second draw) IgG+ contact: individual with history of physical contact with LF case during illness and LASV Ag-, IgM- , IgG+ by ELISA IgG- contact: individual with history of physical contact with LF case during illness and LASV Ag-, IgM-, IgG- by ELISA
Exclusion Criteria	1.1: Privately-operated clinic 1.2 and 1.3: Ag- and IgM- by ELISA	Individual with history of physical contact with LF case during illness and LASV Ag-, IgM+ with less than 4-fold increase in tire at second draw
Enrollment	1.1: 80 PHUs 1.2 and 1.3: Unknown (PHU incidence of LF has not been characterized)	LF case: 320 LASV+ contact: 104 IgG+ contact: 280 IgG- contact: 500
Duration of Enrollment	1.1: Three months 1.2 and 1.3: Four years	4.5 years
Total Duration of Study	4.25 years	5 years
Endpoints/Outcomes	1.1: LF referral rate 1.2: Time to ribavirin treatment 1.3: Death	Metabolic panel test results; Ag, IgM, IgG optical densities; deafness; death
Summary of Data Analysis Plans	1.1: Repeated measures analysis modeling pre-deployment and post-deployment referral rates; linear regression analysis comparing referral rates in PHUs with and without rapid tests. 1.2: Linear regression analysis modeling time to ribavirin treatment in referrals from PHUs with and without rapid tests 1.3: Logistic regression analysis modeling binary death/survival (discharge) in referrals from PHUs with and without rapid tests	Repeated measures analysis modeling outcomes of interest between LASV+ contacts and 3 comparison groups

This is the first submission by Tulane University and the Lassa Fever Program at Kenema Government Hospital to the International Collaborations in Infectious Diseases Research (U19) program.

A Progress Report Publication List is not applicable.

The human subjects research in Projects 1 and 2 meets the definition of "Clinical Research." Therefore, the research protocols and all consent/assent and data forms will be submitted to and approved by the Institutional Review Board (IRB) of Tulane and the Ethics Committees of Sierra Leone prior to implementation of the study. Informed consent will be obtained from all subjects prior to obtaining specimens for research. In some cases, execution of the study may entail testing anonymous sample remainders or serum specimens collected for other purposes. When appropriate, exemption from the requirement of IRB/Ethics Committee approval will be sought and obtained prior to testing the samples.

Patient identification and specimen collection. In Sierra Leone, blood is normally drawn immediately upon clinical suspicion of Lassa fever (which is most often at the time of admission) and then at various intervals thereafter, depending on the clinical course. Samples are collected in 10-ml syringes and stored on wet ice or cold packs and delivered to the KGH Lassa Laboratory. Samples are placed in the refrigerator and allowed to clot and the serum and cells then separated by trained technicians wearing standard personal protective equipment (i.e. masks, gowns, and gloves). Samples are labeled and stored at –20^{o}C until transport can be arranged to KGH (usually <48 hrs), where they are then sent by Ministry of Health-provided ground transport in specialized safety shippers on wet ice or cold packs.

Patient management and infection control. As Lassa fever is endemic in Sierra Leone, the hospitals and health posts in the region habitually see patients with the disease and have standard protocols for infection control. The study proposed here does not consist of a clinical trial and, in most cases, will not seek to obtain specimens from acutely ill patients that would not ordinarily be acquired through the clinically indicated management. However, to insure optimal management of the patient and the safety of the medical staff, with the consent of the hospital administration, patient isolation procedures and facilities will be reviewed and revised or up-graded as necessary. Training in infection control will be conducted and an adequate supply of personal protective equipment and other necessary supplies for the optimal care of patients with Lassa fever, such as syringes and intravenous fluids, will be supplied to each hospital. Although ribavirin is supplied for KGH and other hospitals in catchment areas for Lassa fever by the respective countries' Ministries of Health, supplies are often inadequate. Ribavirin is not approved by the U.S. Food and Drug Administration for the treatment of LF, and we will therefore not purchase or otherwise procure ribavirin. Procurement of ribavirin is at the sole the responsibility of the Sierra Leonean Ministries of Health. All decisions regarding patient care will rest with the local treating physician.

Risks to the subjects

Human subjects involvement and characteristics. Human subjects will be asked to provide blood, and occasionally other biological specimens, such as urine, for diagnostic testing and research. The subject population will be persons hospitalized at the Lassa Ward at KGH and partner institutions, who meet a specific case definition for Lassa fever. Blood specimens may also be obtained from patients during convalescence, as well as from family members or other members of their communities. There are no criteria for exclusion of any sub-population. Blood, blood cell components, serum, plasma, and other biological materials will be periodically sent from Sierra Leone to the other partner laboratories in the Project. All necessary permits will be obtained and biosafety regulations followed in the shipping of any biological specimens.

Sources of materials. Blood and other biological material will be obtained from the human subjects. All of the data obtained, including results of blood tests, will be recorded in both hard copy and electronic databases. Data and test results will be linked to the subject through their name, an assigned unique ID number, and other demographic information. Names will be used only when necessary and of benefit to the patient, such as communicating the patient's relevant clinical laboratory parameters to the treating physician. Only team members directly involved with the study will have access to subject identities. Specimens and data will be collected from the patients at the hospital. Many of the samples collected would normally be obtained for routine diagnostic purposes in the absence of this research project.

Potential risks. The potential risks to subjects in this study are thought to be minimal. The collection of blood may cause minor transient discomfort, but is rarely associated with any serious adverse effects. The amount of blood to be taken will not be enough to cause or exacerbate anemia. Subject names and all information taken will be kept confidential.

Adequacy of protection against risks

Recruitment and informed consent. The study protocol and all consent/assent and data forms will be submitted to and approved by the Institutional Review Boards and ethics committees of all the Project partner institutions/countries prior to implementation of the study. Informed consent will be sought from each patient. The healthcare worker/researcher will describe, in the local African language, the reason and overall plan for the study to all subjects, who will then be asked to participate. Informed oral consent will be recorded. Oral, rather than written,

consent is preferred due to the low literacy rate in the area involved in the study (~20%) and to the cultural hesitance about signing documents, which is a practice normally reserved for only the most serious transactions involving land or inheritance. Parental consent and subject assent will be obtained for persons under 16 years of age. At least two witnesses from the subject's family and two from the research team will be present for the informed consent. The subject's consent/assent and the witnesses' names will be recorded and kept on file.

Protection against risk. All blood drawing will be performed in a sterile fashion by trained personnel. Subjects' information will be kept strictly confidential. Hard-copy databases will be kept in a locked cabinet and electronic copies will be password-protected. A list of researchers who work with the databases will be kept at the laboratory and only those on the list will have access. A reminder of the need to maintain confidentiality will be given at weekly and monthly meetings. We believe these measures are likely to be effective. Although unlikely, subjects who acquire any problems, physical or psychological, related to the study will be instructed to consult with the doctor or nurse. Any charges related to patient care as a direct result of participation in the study will be incurred by the study.

Potential benefits of the proposed research to the subjects.
Participation in the study may afford a direct benefit to some of the subjects through knowledge of the results from the diagnostic and clinical laboratory assays that may help the treating physician better manage the patient's illness. Overall, the benefits to the subjects (independently of the long-term benefit of an increased understanding of the virology, immunology and natural history of Lassa fever seem to be reasonable, considering that the risks from participation in the study are minimal.

Importance of the knowledge to be gained
This study will result in a better understanding of the immunology and pathogenesis of Lassa fever. This will be important for developing countermeasures against these diseases around the world, whether through natural transmission or bioterrorism, including better diagnostic tests, therapies and vaccines.

Human Subjects research conducted in Projects 1 and 2 will involve approximately equal numbers of male and female subjects. Since the subject enrollment will take place in West Africa, 100% of the study population is expected to be black African. There are no exclusions.

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: Aim 1: Evaluation of the deployment of next-generation Lassa fever recombinant antigen immunodiagnostics for point-of-care detection in peripheral health units.

Domestic/Foreign: Foreign

Comments: Hypothesis: Deployment of point-of-care rapid diagnostics to peripheral health units (PHUs) will increase referrals to the LF Ward and reduce time to ribavirin treatment and mortality.

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	500	500	0	0	**1000**
White	0	0	0	0	**0**
More than One Race	0	0	0	0	**0**
Total	**500**	**500**	**0**	**0**	**1000**

Study 1 of 2

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: Aim 3: To utilize ReLASV diagnostics to define correlates of immunity to Lassa fever in patient contacts with little or no disease thereby identifying resistance patterns and susceptible cohorts for Lassa fever.

Domestic/Foreign: Foreign

Comments: Hypothesis: Active LASV+ case surveillance through contact tracing will identify early symptomatic and asymptomatic LASV-infected individuals. These individuals will have different biomarkers than those presenting at KGH through clinic referral and those without active LASV infection.

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	625	625	0	0	**1250**
White	0	0	0	0	**0**
More than One Race	0	0	0	0	**0**
Total	**625**	**625**	**0**	**0**	**1250**

Study 2 of 2

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

Children of all ages will be included in the Human Subjects research conducted in Projects 1 and 2. The research team includes healthcare workers and facilities with extensive experience in dealing with children.

This study involves research on vertebrate animals. *Mastomys natalensis* and other rodents that invade houses in Sierra Leone will be trapped and humanely euthanized during these studies. KGH will obtain an Office of Laboratory Animal Welfare (OLAW) approved Animal Welfare Assurance for Foreign Institutions (Foreign Assurance) before conducting research, research training, and/or biological testing activities involving live, vertebrate animals supported by the Lassa ICIDR grant. The research protocols will also be submitted and approved by the Ethics Committee of the Sierra Leone Ministry of Health and Sanitation, which has statutory authority to regulate and oversee vertebrate animal research.

1. Proposed use of the animals in the work.
Wild small mammals (rodents and shrews) will be trapped in the homes of LF cases and controls. Sherman live-capture traps will be baited and placed inside houses and rodents will be collected within 12 hours of placing the trap. Animals will be identified to the species level by molecular methods and tested for LASV infection or past exposure and genetic subgroups.

2. Justify the use of animals, the choice of species, and the numbers to be used.
None of the rodents involved in this study are in short supply, costly, or will be used in large numbers. We have not identified an endangered or threatened species in this environment. *M. natalensis* is the most ubiquitous rodent species in sub-Subharan Africa. Other rodents are equally important to the research question. The role of other rodent species in transmission of LASV to humans is unknown, but we have identified LASV in other non-reservoir species.

We cannot be definitive about what species will be captured during our studies. Past studies using the same trapping methods proposed here in the same region resulted in a majority of *M. natalensis* (66%). Other animal species trapped included *Rattus rattus* (26%), *Mus* spp. (3%), Crocidura spp. (2%), other rodents (3%). We anticipate 0 to 5 rodents collected for each case or control (see Aim 2) with a maximum of 600 rodents collected.

3. Veterinary care of the animals involved.
Animals will not be housed in a vivarium. No veterinary care will be involved.

4. Discomfort, distress, pain and injury.
Discomfort, distress, pain and injury to rodents will be limited to that which is unavoidable in the conduct of scientifically sound research. Time spent in Sherman traps will be under 12 hours and animals will have food while in cages from the bait, which is not harmful. Rodents will be anesthetized with Isoflurane until unconscious as determined by no reflex response, and tissues will be collected, including whole blood. All methods are recommended by AVMA Guidelines.

5. Euthanasia.
The method of euthanasia is exsanguination while under anesthetic (Isoflurane). Cervical dislocation will be used if exsanguination fails. These methods are consistent with the recommendations of AVMA Guidelines on Euthanasia.

Please refer to the Administrative Core for details regarding Select Agent Research.

As for the other Lassa ICIDR components, the rationale for the Lassa ICIDR Project 1 to be directed by multiple PIDs is guided by the international scope of the collaborative program. We propose that Dr. Jeffery Shaffer, Dr. Lina Moses and Dr. Donald Grant serve as Co-PDs of Lassa ICIDR Project 1. In their roles as Co-PDs, Drs. Shaffer, Moses and Grant will be responsible for the implementation of the specific aims of Lassa ICIDR Project 1, principally Evaluation of 2nd generation recombinant assays as point-of-care diagnostics and surveillance tools for Lassa fever. Dr. Shaffer will serve as the Core contact with Drs. Garry and Khan and will assume fiscal and administrative management of the Core. He will be responsible for submission of annual reports. Decisions on scientific direction and allocation of resources are given to the Core PDs in consultation with Internal and External Advisory Groups. If a potential conflict develops, the Co-PDs shall meet and attempt to resolve the dispute. If they fail to resolve the dispute, the disagreement shall be referred to the EAG. In the event that a PD cannot carry out his duties, a new PD will be recruited as a replacement, subject to the approval of the IAG and EAG.

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All RFA required Letters of Support from Institutional and Government officials are compiled in the Administrative Core section of this application.

Please refer to the Administrative Core for details regarding Resource Sharing.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0537858120000

Legal Name*: TULANE UNIVERSITY
Department:
Division:
Street1*: TULANE UNIVERSITY
Street2: 6823 ST. CHARLES AVE
City*: NEW ORLEANS
County:
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 701180000

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
Ms.	Kathleen	M	Kozar	

Position/Title: Director
Street1*: 1430 Tulane Avenue, Ep-15
Street2:
City*: New Orleans
County: Orleans
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 701122613
Phone Number*: 5049885613 Fax Number: 5049881748 Email: elecnot@tulane.edu

7. TYPE OF APPLICANT*

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Project 2. Expansion of clinical research capacity at Kenema Government Hospital

12. PROPOSED PROJECT

Start Date*	Ending Date*
01/01/2015	12/31/2019

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	TULANE UNIVERSITY
Duns Number:	0537858120000
Street1*:	TULANE UNIVERSITY
Street2:	6823 ST. CHARLES AVE
City*:	NEW ORLEANS
County:	
State*:	LA: Louisiana
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	701180000
Project/Performance Site Congressional District*:	LA-002

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Kenema Government Hospital
DUNS Number:	8505106210000
Street1*:	1 Combema Road
Street2:	
City*:	Kenema
County:	
State*:	
Province:	
Country*:	SLE: SIERRA LEONE
Zip / Postal Code*:	
Project/Performance Site Congressional District*:	

File Name

Additional Location(s)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ○ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ● No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ○ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ● **Yes** ○ **No**

6.a. If yes, identify countries: Sierra Leone

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_2_Abstract.pdf
8. Project Narrative*	Pjt_2_Narrative.pdf
9. Bibliography & References Cited	Pjt_2_References.pdf
10.Facilities & Other Resources	Facilities__ICIDR_.pdf
11.Equipment	Lassa_ICIDR_Equipment.pdf

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

Project 2. Expansion of clinical research capacity at Kenema Government Hospital

Lassa virus (LASV), a member of the family *Arenaviridae*, is the etiologic agent of Lassa fever (LF), a severe and often fatal hemorrhagic illness. It has been reported throughout West Africa from Guinea in the west to Nigeria in the east. Humans contract LASV primarily via direct or indirect contact with body fluids of rodents of the genus *Mastomys*, the natural reservoir. Nosocomial transmission can lead to large hospital outbreaks. The possibility of aerosol transmission and the lack of a vaccine have led to its classification as a BSL-4 and Category A Select Agent. Previous studies performed in the 1980's estimated up to 300,000 human LASV infections per year with 5,000 deaths per year. However, the natural history and mortality rate of disease remain poorly defined. Since these landmark studies were performed, significant advances have been made in LF clinical diagnostics as well as the laboratory capacity in endemic areas. Using newly developed Ag, IgG and IgM recombinant ELISAs (rELISA) and improved capacity at the site, we are now able to accurately study viremia and the immune response. This proposal will test the hypothesis: Development of IgM, but not a cellular inflammatory response, and decline in viremia during the early stages of symptomatic LF disease decreases mortality.

Aim 1 is too identify clinical and virological determinants of Lassa fever outcome in a cohort of symptomatic Lassa fever patients presenting to Kenema Government Hospital. Previous studies have identified several clinical features and clinical laboratory parameters that predict a poor prognosis. However, no study has determined if degree of viremia correlates with clinical features or laboratory test abnormalities. We will test the hypothesis: Prolonged viremia correlates with disease progression and increased mortality. We will identify clinical and laboratory diagnostic and prognostic risk factors for LF and correlate them with viremia and risk of mortality. This aim will establish the natural history of LF disease and determine if the time course can be divided into distinct stages as previously suggested. Aim 2 is to identify biomarkers of infection and mortality. Recent development of rLASV IgM and IgG ELISAs as well as increased capacity at the KGH Lassa Fever Laboratory provide a unique opportunity to identify specific immune correlates with disease severity and risk of mortality. We will test the hypothesis: Delayed development of the humoral and an increased TH1 inflammatory immune response correlates with increased mortality. We will correlate specific humoral and cytokine profiles with disease progression and outcome. These studies are critical for the design of future interventional trials and the development of therapies for LF and other viral hemorrhagic fevers. Aim 3 will initiate Clinical trial capacity training. Over the past decade, a significant amount has been learned about the pathogenesis of LF and the human immune response. Trials are now needed to test existing and novel therapies for this disease. The objective of this aim is to build on previously funded capacity at the site and train critical team members in the essential elements needed to conduct clinical trials. This training is critical if the KGH site is to continue to contribute to our knowledge of and management strategies for VHFs.

The objective of this proposal is to define the immune response to Lassa fever in symptomatic, hospitalized patients and to improve the clinical research capacity of this unique site. The long-term goal of this project is to further develop the highly productive partnership between Tulane University and the Sierra Leone Lassa Fever Project. This work is vital to the future design and implementation of definitive diagnostic and therapeutic trials.

Clinical and Laboratory resources in Kenema, Sierra Leone (ICIDR Projects 1 and 2)

Kenema Government Hospital (KGH) in Sierra Leone, West Africa is a 350-bed facility situated in the heart of the region with the world's highest incidence of Lassa fever. Because of the importance of Lassa fever as a bioterrorism and public health threat, KGH has developed an advanced clinical and laboratory research capacity. To our knowledge, this is one of two facilities continuously operated and dedicated to the care of patients infected with a Category A Select Agent anywhere in the world. The KGH Laboratory has the capacity and quality control to perform diagnostic testing for Lassa fever and is the only facility with laboratory testing and clinical treatment of Lassa fever in the entire country. In addition, the *Central Public Health Laboratory* (CPHL) in Liberia is accustomed to receiving samples from suspected cases of Lassa fever, safely packaging them (specific training on this matter has been conducted), and delivering them by ground transport to Kenema for diagnostic testing. Almost every other week, samples are sent from Liberia to Kenema.

Fig A. Sample Processing in the Kenema Government Hospital Lassa Laboratory. Samples are manipulated in class II biosafety cabinets by personnel wearing full personnel protective materials.

The enhanced physical and organizational infrastructure presents an unparalleled opportunity to access and test clinical samples in areas where Lassa fever is a frequent occurrence. The necessary administrative infrastructure for research on Lassa fever in Sierra Leone is also well established. Sierra Leone has an ethics committee to review and approve research protocols and Federal Wide Assurances from the U.S. Department of Health and Human Services. Ultimately, the high incidence of Lassa fever, along with the unique infrastructure and resources of our established public health and research network in West Africa offer unique opportunities to conduct detailed and integrated studies on a VHF and Category A Select Agent.

The Lassa Laboratory at KGH.

The KGH Lassa Laboratory is located on the grounds of the hospital, but in a stand-alone building constructed in 2005. Samples are manipulated in class II biosafety cabinets by extensively trained personnel wearing full personal protective materials (gowns, gloves, eye protection, mask and rubber boots) (Fig. A). The laboratory is comprised of an approximately 5,500 square foot building divided into a general clinical laboratory for routine diagnostics that services the entire hospital and a 700 square-foot specialized suite for manipulation of samples from suspected cases of LF (Fig. B). The building is equipped with redundant power sources, including municipal power (which is extremely sporadic), a solar power system (panels, batteries, controllers and inverters, and 100, 16 and 6 kilovolt generators. ELISA (antigen and IgM and IgG antibody), and lateral flow rapid test for Lassa fever are performed here. Access to the building and the BSL-3 suite is restricted. Negative airflow is maintained. The laboratory also possesses equipment and trained personnel for RT-PCR and cell culture (with separate PCR and cell culture suites – Fig. C). The cell culture suites are used to prepare sera and PBMCs for shipment back to Tulane for further

Fig. B. The Kenema Government Hospital Lassa Laboratory. The solar panels provide essential power for refrigerators, freezers and other essential instruments.

analysis and isolation of human monoclonal antibodies to Lassa virus. Established biosafety and biosecurity guidelines are maintained, with oversight by the Sierra Leone Ministry of Health and Sanitation, WHO and Tulane. The KGH Lassa Laboratory has undergone site review by NIH Program Officers and found to be acceptable. Mr. Augustine Goba, the KGH Lassa Laboratory Director, has been actively involved in Lassa fever research since the 1980's. Mr Goba supervises three technicians.

Fig. C. The structure shown, which is adjacent to the KGH laboratory, is constructed from prefabricated office containers donated by the United Nations, which have been subsequently refitted to serve as PCR clean rooms and cell culture suites. A. 2006 Toyota Land Cruiser (shown) is available to partly support the proposed project, but will soon require replacement.

The Lassa Ward at KGH

KGH current maintains a year-round 25-bed ward for the care of patients with LF at which up to 600 suspected cases seen yearly. The ward is staffed with a full-time team of doctors, nurses, and cleaners. The staff has extensive training and experience treating and caring for LF patients and the majority have over a decade of experience with LF patients. Standard guidelines for the isolation and management of patients with viral hemorrhagic fevers (VHFs) are maintained (65). All personnel entering patient care areas are required to wear appropriate personal protective equipment (PPE): surgical scrubs and gown, rubber apron, double gloves, rubber boots, mask and face shield. Personnel must wash their hands with a bleach solution after patient contact. The Lassa Ward has two dedicated ambulances for patient transport. All drivers are trained in the use of PPE. The patient care area of the ambulance and ward as well as all durable equipment are washed with a bleached solution after use by trained cleaners.

The KGH team has broken ground on a new 48-bed Lassa Ward that will replace this historic, but timeworn facility (Fig. D). Funding for the new Lassa Ward was provided by from the Naval Facilities Engineering Command (NAVFAC), heading the medical diplomacy missions of DoD, with technical support from Tulane and the Viral Hemorrhagic Fever Consortium (www.VHFC.org).

Kenema Government Hospital Lassa Fever Outreach Team

The outreach team at KGH is a highly experienced group of community outreach workers (Figs E and F). The team is heavily integrated into Sierra Leone's highly structured health surveillance system, working in close partnership with district medical and surveillance officers. The outreach team conducts LF case investigations country-wide which also includes contact tracing and updating local stakeholders. The team also is heavily involved in community education for the prevention and early detection of LF including implementation of rodent control interventions and sanitation marketing. The team is well-versed in survey methods including sampling strategies, questionnaire administration, and collection of blood and other specimens in community settings. and his knowledge of the communities in eastern Sierra Leone is extensive. The team currently consists of two junior and one experienced member in addtion to their supervisor. If project is funded, one additional staff will be added. Team members speak the majority of dialects found in Sierra Leone including Krio, Mende, Temne, and Kono. The outreach team supervisor, Lansana Kanneh has been with the LF outreach team since 1994 and was appointed supervisor in 2013. Mr. Kanneh is a vibrant public speaker. He is currently working on a diplomma in public health with support from Tulane University.

Fig. D. The Lassa Ward. The current Lassa Ward is a 25-bed facility with a full-time clinical and nursing staff dedicated to the treatment of Lassa fever patients (top left). This facility will be replaced with a much-needed 48-bed facility (funded by NAVFAC) with construction by Fajaha International Construction expected to be completed by Q3 2014. The new ward will contain offices, a pharmacy, medical records room as well as high and low containment wards. The rooms were specifically designed to deal with infection control issues associated with VHFs. Layout of the facility and example of current progress (middle panels) and details of the construction plan (lower panel) were developed with technical assistance from Jason Moses (Jason Moses Projects, NYC) and the VHFC.

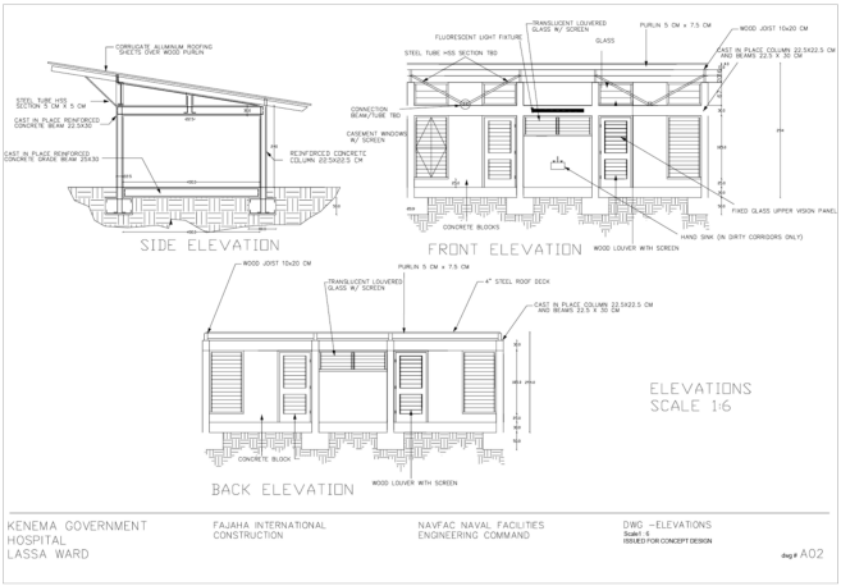

Fig. E. Community meetings with the KGH LF Outreach Team are done in evenings when subsistence farmers are back from their fields. . In addition to case investigations, the team also traps and distributes rodent control products to case households to prevent additional infections.

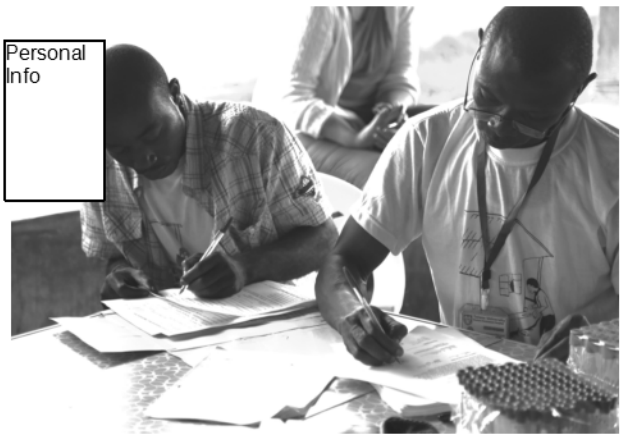

Fig. F. Mr. Lansana Kanneh, outreach team supervisor, performing informed consent and blood draws. Screening of normal or afebrile population clusters and identification of sub-clinically infected subjects in Project 1.

Kenema Government Hospital Lassa Fever EcologyTeam

The KGH ecology team has extensive knowledge on trapping of small mammals for zoonotic disease research (Figs. G and H). The team has expertise in field survey methods, live-capture trapping and sample preservation and storage. They also are experience rodent control and hygiene experts and provide a valuable resource for the development of LF prevention programs.

Fig. G. James Koninga (l) Kandeh Kargbo (m) and Lina Moses (r) during the necropsy of a juvenile *Mastomys natalensis*. In addition to biological specimens for virological testing, the team collects morphometric data for ecology focused research.

Fig. H. James Koninga (r) sets a Sherman live-capture trap in a corn field targeting *Mastomys natalensis.* Willie Robert (l) records position on transect line and documents GPS location for spatial studies.

Supervisor, Mr, James Koninga, has participated in collection of small mammals for zoonotic disease research since 1979 when he was trained by Centers for Disease Control and Prevention personnel during the first documented community epidemic of Lassa fever. Mr. Koninga has since participated in animal surveys during Ebola outbreaks and has received voucher preparation training from the University of New Mexico. Mr. Koninga is an expert in the safe handling and collection of biological materials from wild animals and ensures a high level of biosafety and sample quality assurance while in the field. Mr. Koninga supervises two junior ecologists for Tulane's research projects.

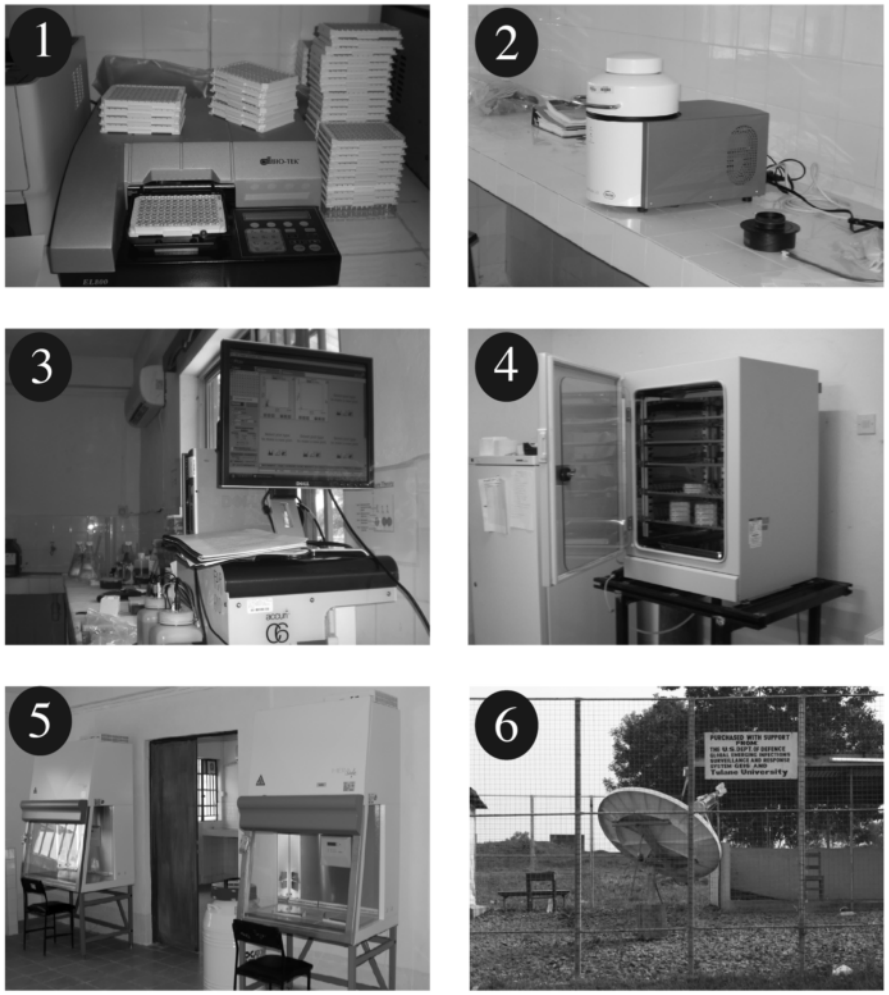

Fig α. Roche LightCycler 2.0 – real-time PCR machine in the KGH laboratory.

Kenema Government Hospital, Sierra Leone

Pertinent equipment items in the KGH Lassa Laboratory are shown in Figure α, including ELISA plate readers and washers (1), light-cycler real-time PCR machine (2), Accuri C6 flow cytometer (3), CO_2 incubator (4) class II biosafety cabinets (5), satellite internet communication system (6). The Lassa Laboratory also has LN2 tanks, a 4 liter per day LN2 generator, ELISpot reader, fluorescent microscope, conventional microscopes, table top centrifuges, microfuges, water baths, instrument sterilizer, solar and electric powered freezers, refrigerators, freezers, Millipore Direct Q water purification system, pH meter, vortex, balances and vacuum pumps.

Irrua Specialist Teaching Hospital, Irrua, Nigeria.

The laboratory facility is equipped with equipment to process and store blood samples and specimens, as well as RT- PCR and ELISA diagnosis of Lassa fever. These equipment include: 1 PCT-200 Thermocycler, 1 Sigma 415K Tabletop cold centrifuge, 1 Eppendorf cold microcentrifuge tube, 1 gel documentation system, Electrophoresis apparatus, 1 Barcoder and Barcoder stand, Micropipettes, Water distiller, 1 hood, 3 freezers, 1 fridge, 1 desktop computer, 1 autoclave and 1 digital stirred water bath.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: John Middle Name Scribner Last Name*: Schieffelin Suffix: M.D.

Position/Title*:	Assistant Professor of Pediatrics
Organization Name*:	Tulane University
Department:	
Division:	
Street1*:	1430 Tulane Ave
Street2:	TB-8
City*:	New Orleans
County:	
State*:	LA: Louisiana
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	701120000

Phone Number*: 5049885117 Fax Number: 5049882613 E-Mail*: jschieff@tulane.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: Degree Year:

File Name

Attach Biographical Sketch*:

Attach Current & Pending Support:

Funding Opportunity Number: RFA-AI-14-002 . Received Date: 2014-03-07T14:26:44.000-05:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Shiek Middle Name Humarr Last Name*: Khan Suffix: M.D.

Position/Title*: Director
Organization Name*: Kenema Givernment Hspital
Department: Lassa Fever Program
Division:
Street1*: 1 Combema Road
Street2:
City*: Kenema
County:
State*:
Province:
Country*: SLE: SIERRA LEONE
Zip / Postal Code*:

Phone Number*: 232 78 627 621 Fax Number: E-Mail*: Personal Info

Credential, e.g., agency login:

Project Role*: Co-PD/PI Other Project Role Category:

Degree Type: MBChB Degree Year:

File Name

Attach Biographical Sketch*:

Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: First Name*: Jeffrey Middle Name George Last Name*: Shaffer Suffix:

Position/Title*: Assistant Professor
Organization Name*: Tulane University
Department: Biostatistics
Division: Public Health/Trop. Medicine
Street1*: 1440 Canal Street
Street2:
City*: New Orleans
County:
State*: LA: Louisiana
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 701122613

Phone Number*: 5044328658 Fax Number: E-Mail*: jshaffer@tulane.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: BS, MS, PhD Degree Year: 1999, 2001, 2007

File Name

Attach Biographical Sketch*:

Attach Current & Pending Support:

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Prof.	John	S.	Schieffelin	M.D.	Co-PD	Institutional Base Salary	EFFORT			34,146.00	5,976.00	40,122.00
2.	Prof.	Jeffrey	G.	Shaffer	Ph.D	Co-Investigator					7,315.00	1,258.00	8,573.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **48,695.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**48,695.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
1. Chison 8300 Portable Ultrasound	4,000.00
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**4,000.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	36,000.00
Total Travel Cost	**40,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT11602615 Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	42,705.00
2. Publication Costs	2,600.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	72,000.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**117,305.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**210,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	134,000.00	67,670.00
		Total Indirect Costs	**67,670.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**277,670.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_2_Budget_Justification_ICIDR_v1.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Prof.	John	S.	Schieffelin	M.D.	Co-PD	Institutional Base Salary	EFFORT			34,146.00	5,976.00	40,122.00
2.	Prof.	Jeffrey	G.	Shaffer	Ph.D	Co-Investigator					7,315.00	1,258.00	8,573.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **48,695.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**48,695.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	36,000.00
Total Travel Cost	**40,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT11602615 Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2016 **End Date*:** 12-31-2016 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	46,705.00
2. Publication Costs	2,600.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	72,000.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**121,305.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**210,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	138,000.00	69,690.00
		Total Indirect Costs	**69,690.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**279,690.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_2_Budget_Justification_ICIDR_v1.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2017 **End Date*:** 12-31-2017 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Prof.	John	S.	Schieffelin	M.D.	Co-PD	Institutional Base Salary	EFFORT			34,146.00	5,976.00	40,122.00
2.	Prof.	Jeffrey	G.	Shaffer	Ph.D	Co-Investigator					7,315.00	1,258.00	8,573.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **48,695.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**48,695.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2017 **End Date*:** 12-31-2017 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	36,000.00
Total Travel Cost	**40,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2017 **End Date*:** 12-31-2017 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	46,705.00
2. Publication Costs	2,600.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	72,000.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**121,305.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**210,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	138,000.00	69,690.00
		Total Indirect Costs	**69,690.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**279,690.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_2_Budget_Justification_ICIDR_v1.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Prof.	John	S.	Schieffelin	M.D.	Co-PD	Institutional Base Salary	EFFORT			34,146.00	5,976.00	40,122.00
2.	Prof.	Jeffrey	G.	Shaffer	Ph.D	Co-Investigator					7,315.00	1,258.00	8,573.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **48,695.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**48,695.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	36,000.00
Total Travel Cost	**40,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT11602615 Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2018 **End Date*:** 12-31-2018 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	46,705.00
2. Publication Costs	2,600.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	72,000.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**121,305.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**210,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	138,000.00	69,690.00
		Total Indirect Costs	**69,690.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**279,690.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_2_Budget_Justification_ICIDR_v1.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2019 **End Date*:** 12-31-2019 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Prof.	John	S.	Schieffelin	M.D.	Co-PD	Institutional Base Salary	EFFORT			34,146.00	5,976.00	40,122.00
2.	Prof.	Jeffrey	G.	Shaffer	Ph.D	Co-Investigator					7,315.00	1,258.00	8,573.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **48,695.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**48,695.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2019 **End Date*:** 12-31-2019 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
1. Chison 8300 Portable Ultrasound	4,000.00
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**4,000.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	36,000.00
Total Travel Cost	**40,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0537858120000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY

Start Date*: 01-01-2019 **End Date*:** 12-31-2019 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	42,705.00
2. Publication Costs	2,600.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	72,000.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**117,305.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**210,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	134,000.00	67,670.00
		Total Indirect Costs	**67,670.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**277,670.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_2_Budget_Justification_ICIDR_v1.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Senior/Key Personnel:

John Schieffelin, MD, Project Co-Director EFFORT is Assistant Professor of Pediatrics and Internal Medicine at Tulane. He has been co-investigator on other Lassa fever projects funded by the NIH in Sierra Leone for over three years. He is also the clinical director of the Viral Hemorrhagic Fever Consortium, a partnership of research institutes whose mission is to promote global health and safety by creating new products to diagnose, treat and significantly reduce the incidence and mortality rate of viral hemorrhagic fevers. He has extensive experience managing clinical research projects at the study site, Kenema Government Hospital in Kenema, Sierra Leone. Dr. Schieffelin will be responsible with Dr. S. Humarr Khan for the overall administration and direction of Project 2.

Jeffrey G. Shaffer, PhD, Co-Investigator (Data Manager), EFFORT is a Research Assistant Professor in the Department of Biostatistics and Bioinformatics at the Tulane University School of Public Health and Tropical Medicine. Dr. Shaffer is an experienced statistician and data manager and will coordinate the collection and statistical analysis of the data. He has worked with the Tulane University Lassa Fever Program for over 5 years. He maintains a database of all clinical and laboratory data collected at the Kenema site. He has significantly contributed to the study design and power and sample size calculations for proposed Project 2.

Supplies:

Blood collection supplies	$4,000
Lassa ELISA reagents (2nd Gen)	$10,000
Point of care clinical tests (2nd Gen)	$8,000
Clinical lab reagents	$10,000
Research Lab reagents	$6,405
Biosafety supplies (PPE)	$4,300
Total	$42,705

Significant costs are incurred during the care of Lassa fever patients. Although the Ministry of Health operates and maintains the Lassa Fever Ward at Kenema Government Hospital, additional supplies and staff support are necessary for clinical research. Vital supplies such as gloves, gowns, masks and other personal protective equipment are not reliably available in Sierra Leone. They are essential for protection of staff caring for patients suspected to have Lassa fever. Additionally, basic medical supplies are not always available in Sierra Leone such as thermometers, blood pressure cuffs, stethoscopes, etc. which are required for the clinical studies No funds will be used for basic clinical care or investigational drug purchases. Results with the second generation Lassa fever diagnostics will be compared to the first generation assays currently employed.

NOTE: All these supplies will be utilized at Kenema Government Hospital. Our practice is to purchase these supplies in New Orleans and then transport them to Kenema in boxes or trunks as excess baggage [each traveler can transport up to 9 “extra” pieces of luggage]. We also anticipate and have budgeted under the Administration Core for transport of one ocean-going container to KGH in each project year. The team is experienced in the logistics needed to maintain the laboratory and clinical operations at KGH. A limited number of supplies can be obtained locally and these are included under the consortium budget.

Publication Costs: $2,600

We anticipate that the year’s activities will result in at least one peer-reviewed publication, which will be submitted to an open access journal. Publication fees for a typical open access journal such as PLoS Neglected Tropical Diseases are approximately $2600.

Equipment: $4,000

A portable ultrasound is needed for more advanced medical management of Lassa fever patients with vascular leak syndrome and shock. Other interventional strategies are not appropriate for this setting and require invasive techniques that put health care providers at risk. Per the manufacturer, the Chison 8300 portable ultrasound is appropriate for resource poor settings and for practitioners with limited ultrasound experience. It has an average life expectancy of four years. Therefore, we will budget for a machine in year 1 as well as year 5.

Domestic Travel: $4,000
As specified in the RFA, funds are requested for one of the Co-PIs (Dr. Schieffelin or Dr. Shaffer) to travel to the ICIDR Kick-Off Meeting within 3 months of award. In future years one of the Co-PIs will attend the Annual Programmatic Meeting.

International Travel: $36,000
Airfare to and from Sierra Leone costs approximately $2,500. In-country travel, hotel and per diem cost an additional $500 per trip. We are budgeting for Drs. Schieffelin and Shaffer, who will also serve with Dr. Garry as overall Lassa ICIDR Co-PIs, to each oversee research activity at the Sierra Leone site 4 times per year (total time for US Co-PIs in Sierra Leone exceeds RFA requirements for total US PI time spent at the International site). In addition, as discussed under Specific Aim 4, KGH personnel will travel to Tulane University or other locations in the United States for short periods of intense training in laboratory techniques, ultrasound training bioethics, regulatory requirements and data management.

Indirect Costs:
The Indirect Cost Rate for the Tulane University School of Medicine (TUHSC) is 50.5% (Modified Total Direct). Indirect costs are not applied to equipment or Consortium costs.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**243,475.00**
Section B, Other Personnel		**0.00**
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		**243,475.00**
Section C, Equipment		**8,000.00**
Section D, Travel		**200,000.00**
1. Domestic	20,000.00	
2. Foreign	180,000.00	
Section E, Participant/Trainee Support Costs		**0.00**
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		**598,525.00**
1. Materials and Supplies	225,525.00	
2. Publication Costs	13,000.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	360,000.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		**1,050,000.00**
Section H, Indirect Costs		**344,410.00**
Section I, Total Direct and Indirect Costs (G + H)		**1,394,410.00**
Section J, Fee		**0.00**

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Sheik	Humarr	Khan	M.D.	Co-PD	Institutional Base Salary	EFFORT			9,000.00	1,350.00	10,350.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **10,350.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	Nurses	Institutional Base Salary			9,000.00	1,350.00	10,350.00
3	Laboratory				6,000.00	900.00	6,900.00
3	Support staff				1,500.00	225.00	1,725.00
2	Drivers				4,000.00	600.00	4,600.00
4	Outreach Team				4,000.00	600.00	4,600.00
16	**Total Number Other Personnel**					**Total Other Personnel**	**28,175.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**38,525.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	12,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**12,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		6,000.00
2. Stipends		3,000.00
3. Travel		500.00
4. Subsistence		500.00
5. Other:		
5 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**10,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	3,475.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Lassa Ward generator fuel	8,000.00
Total Other Direct Costs	**11,475.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**72,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**72,000.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_2_KGH_Budget_Justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1. Dr.	Sheik	Humarr	Khan	M.D.	Co-PD	Institutional Base Salary	EFFORT			9,000.00	1,350.00	10,350.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **10,350.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	Nurses	EFFORT			9,000.00	1,350.00	10,350.00
3	Laboratory				6,000.00	900.00	6,900.00
3	Support staff				1,500.00	225.00	1,725.00
2	Drivers				4,000.00	600.00	4,600.00
4	Outreach Team				4,000.00	600.00	4,600.00
16	**Total Number Other Personnel**					**Total Other Personnel**	**28,175.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**38,525.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	12,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**12,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		6,000.00
2. Stipends		3,000.00
3. Travel		500.00
4. Subsistence		5.00
5. Other:		
5 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**9,505.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	3,475.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Lassa Ward generator fuel	8,000.00
Total Other Direct Costs	**11,475.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**71,505.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**71,505.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_2_KGH_Budget_Justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Sheik	Humarr	Khan	M.D.	Co-PD	Institutional Base Salary	EFFORT			9,000.00	1,350.00	10,350.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **10,350.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	Nurses	EFFORT			9,000.00	1,350.00	10,350.00
3	Laboratory				6,000.00	900.00	6,900.00
3	Support staff				1,500.00	225.00	1,725.00
2	Drivers				4,000.00	600.00	4,600.00
4	Outreach Team				4,000.00	600.00	4,600.00
16	**Total Number Other Personnel**					**Total Other Personnel**	**28,175.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**38,525.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	12,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**12,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		6,000.00
2. Stipends		3,000.00
3. Travel		500.00
4. Subsistence		500.00
5. Other:		
5 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**10,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	3,475.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Lassa Ward generator fuel	8,000.00
Total Other Direct Costs	**11,475.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**72,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**72,000.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Pjt_2_KGH_Budget_Justification_REVISED.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Sheik	Humarr	Khan	M.D.	Co-PD	Institutional Base Salary	EFFORT			9,000.00	1,350.00	10,350.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **10,350.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	Nurses	EFFORT			9,000.00	1,350.00	10,350.00
3	Laboratory				6,000.00	900.00	6,900.00
3	Support staff				1,500.00	225.00	1,725.00
2	Drivers				4,000.00	600.00	4,600.00
4	Outreach Team				4,000.00	600.00	4,600.00
16	**Total Number Other Personnel**					**Total Other Personnel**	**28,175.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**38,525.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	12,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**12,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		6,000.00
2. Stipends		3,000.00
3. Travel		500.00
4. Subsistence		500.00
5. Other:		
5 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**10,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	3,475.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Lassa Ward generator fuel	8,000.00
Total Other Direct Costs	**11,475.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**72,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**72,000.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_2_KGH_Budget_Justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Sheik	Humarr	Khan	M.D.	Co-PD	Institutional Base Salary	EFFORT			9,000.00	1,350.00	10,350.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **10,350.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	Nurses	EFFORT			9,000.00	1,350.00	10,350.00
3	Laboratory				6,000.00	900.00	6,900.00
3	Support staff				1,500.00	225.00	1,725.00
2	Drivers				4,000.00	600.00	4,600.00
4	Outreach Team				4,000.00	600.00	4,600.00
16	**Total Number Other Personnel**					**Total Other Personnel**	**28,175.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**38,525.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	12,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**12,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		6,000.00
2. Stipends		3,000.00
3. Travel		500.00
4. Subsistence		500.00
5. Other:		
5 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**10,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 01-01-2015 **End Date*:** 12-31-2015 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	3,475.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Lassa Ward generator fuel	8,000.00
Total Other Direct Costs	**11,475.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**72,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**72,000.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Pjt_2_KGH_Budget_Justification_REVISED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Senior/Key Personnel:
Sheik Humarr Khan, M.B.Ch.B., Co-Director of Project 2 EFFORT is the Director of the National Lassa Fever Program of the Ministry of Health and Sanitation (MOHS) in Sierra Leone. He is permanently assigned to Kenema Government Hospital. He has over 10 years experience treating Lassa patients and recently completed a residency in Internal Medicine at Korle Bu Teaching Hospital in Ghana Dr. Khan is responsible for patient admission, testing and medical care on the Lassa Fever Ward. As Director of the National Lassa Fever Program, he also oversees activities of the Lassa Fever Laboratory and Outreach Teams.

Laboratory:
Augustine J. Goba, Senior Laboratory Technician, Director of the Lassa Fever Laboratory. Mr. Goba has over 25 years of experience as a Senior Laboratory Technician having served as Chief Technician on the Centers for Disease Control and Prevention (CDC) Lassa Fever Project in Kenema, Sierra Leone from 1987 to 1994. During the Civil War in Sierra Leone, Mr. Goba worked as a Chief Laboratory Technician in the CDC Guinea Lassa Fever Project, N.'Zérékoré, Guinea before returning to Kenema after the War in 2005 to resume his position at the KGH Laboratory. Mr. Goba will be primarily responsible for performing the Lassa virus ELISAs and oversees routine hematology and chemistry testing of samples in the Lassa Fever Laboratory. He also supervises two medical technologists each with over five years experience, **Mohamed Fullah** and **Mambu Momoh**.

Nurses:
Mrs. Mbalu Fonnie, senior matron of nursing at KGH is a licensed nurse midwife and is the nursing supervisor of the KGH Lassa Fever Ward. She has over 30 years experience treating Lassa fever and specializes in the management of severe Lassa cases in pregnant women. Mrs. Fonnie began her career working at Nixon Methodist Hospital in Segbwema, Sierra Leone and participated in research trials conducted by the Centers for Disease Control. She supervises a nursing staff of nine nurses and nurse aids.

Ms. Veronica Koroma, assistant nursing supervisor and midwife, has over 15 years experience treating Lassa fever patients. She has devoted much of her life to the treatment and research of Lassa fever. Ms. Koroma is responsible for ensuring that nursing coverage is adequate and oversees the care of all acutely ill patients on a day-to-day basis.

Mr. Michael Gbakie is a registered nurse in Sierra Leone and serves as the Clinical Research Coordinator for the Tulane Lassa Fever Program. He has over 10 years experience with management and research of Lassa fever. Mr. Gbakie is responsible for the quality control of the onsite research. In this position, he is for ensures that all enrolled subjects are consented properly and that all case report forms are completed appropriately. In addition, he supervises a laboratory technician, **Sidiki Safa**, and works with the nursing staff to ensure that all laboratory testing is done per protocol. Mr. Safa also has over 20 years experience collecting blood samples from Lassa fever patients and has been a critical staff member for many years.

Support Staff:
Victor Lungay will serve Database Manager/Statistician and will oversee data entry, statistical analysis, and interpretation as he does currently for WHO-sponsored projects at KGH. Two part-time cleaners work in the Lassa Laboratory and Lassa Laboratory.

Drivers:
Drivers, mechanics (M Sow, M Fomgbeh EFF ORT) will support the Outreach and Ecology teams.

Outreach Team:
Lansana Kanneh a Sierra Leonean has 7 years experience in research projects with Tulane University and 20 years of experience on LF community education and surveillance. He is fluent in Mende and Krio and is extremely knowledgeable about the distribution of Lassa fever cases and geography of Sierra Leone. Mr. Kanneh, as supervisor of the Lassa Fever Outreach Team oversees case investigations and contact tracing of all Lassa fever cases diagnosed at IGH. In addition, he oversees and coordinates follow-up sample collections from Lassa patients discharged from the Lassa Ward. Mr. Kanneh will supervises a team of thee Outreach Team members.

Supplies: $3,475 per year
Additional supplies and staff support are necessary for clinical research. Extra personnel are required for clinical research at this site including nurses, study coordinators, ambulance drivers and outreach workers for case investigation and patient follow-up visits. No funds will be used for routine clinical support or investigational drug purchases. Materials and supplies are needed for production of data collection forms, electronic tablets for data collection, GPS units, field team supplies.

Domestic Travel:
Project 2 will require visits to locations outside of Kenema Government Hospital each year, requiring logistic support and fuel costs. Several of the regions are a day's drive from Kenema Government Hospital. Housing costs will be re-imbursed will be given to members of the Lassa Fever Program Outreach Team who must spend several days in the field for these visits.

Training:
Funds are budgeted in each year for tuition and fees and other costs related to Training Programs, materials and travel costs for Sierra Leonean staff that will be administered in Sierra Leone institutions, including the University of Sierra Leone (Freetown) and Eastern Polytechnic Institute (Kenema).

Other Expenses:
Town power in Kenema is highly unreliable. Town power is available about 5% of the time in Kenema, and even during those periods frequent intermittent or sustained power outages occur. Although the KGH Lassa Laboratory has limited solar power capability it is not sufficient to supply the power needs of the Lassa Ward, which relies on a generator. $8000 per year is requested for diesel fuel.

Indirect Costs:
None requested.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**51,750.00**
Section B, Other Personnel		**140,875.00**
Total Number Other Personnel	80	
Total Salary, Wages and Fringe Benefits (A+B)		**192,625.00**
Section C, Equipment		**0.00**
Section D, Travel		**60,000.00**
1. Domestic	60,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		**49,505.00**
1. Tuition/Fees/Health Insurance	30,000.00	
2. Stipends	15,000.00	
3. Travel	2,500.00	
4. Subsistence	2,005.00	
5. Other	0.00	
6. Number of Participants/Trainees	25	
Section F, Other Direct Costs		**57,375.00**
1. Materials and Supplies	17,375.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	40,000.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		**359,505.00**
Section H, Indirect Costs		**0.00**
Section I, Total Direct and Indirect Costs (G + H)		**359,505.00**
Section J, Fee		**0.00**

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:	Dr.
First Name*:	John
Middle Name:	Scribner
Last Name*:	Schieffelin
Suffix:	M.D.

2. Human Subjects

Clinical Trial?	● No	○ Yes
Agency-Defined Phase III Clinical Trial?*	○ No	○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ○ No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Project_2_Aims.pdf
3. Research Strategy*	Project_2_Research_Strategy_final.pdf
4. Progress Report Publication List	Progress_Report_Publication_List.pdf
Human Subjects Sections	
5. Protection of Human Subjects	Human_subjects.pdf
6. Inclusion of Women and Minorities	Women.pdf
7. Inclusion of Children	Children.pdf
Other Research Plan Sections	
8. Vertebrate Animals	Project_2_no_Vert_Animals.pdf
9. Select Agent Research	Admin_Core_manage_Select_Agent_Research.pdf
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	Admin_Core_manage_Consortium_Agreement.pdf
12. Letters of Support	LoS_in_Admin_Core.pdf
13. Resource Sharing Plan(s)	Admin_Core_manage_Res_Sharing.pdf
Appendix (if applicable)	
14. Appendix	

Tracking Number: GRANT11602615 Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

Project 2. Expansion of clinical research capacity at Kenema Government Hospital

Lassa virus (LASV), a member of the family *Arenaviridae*, is the etiologic agent of Lassa fever (LF), a severe and often fatal hemorrhagic illness. It has been reported throughout West Africa from Guinea in the west to Nigeria in the east. Humans contract LASV primarily via direct or indirect contact with body fluids of rodents of the genus *Mastomys*, the natural reservoir. Nosocomial transmission can lead to large hospital outbreaks. The possibility of aerosol transmission and the lack of a vaccine have led to its classification as a BSL-4 and Category A Select Agent. Previous studies performed in the 1980's estimated up to 300,000 human LASV infections per year with 5,000 deaths per year. However, the natural history and mortality rate of disease remain poorly defined. Since these landmark studies were performed, significant advances have been made in LF clinical diagnostics as well as the laboratory capacity in endemic areas. Using newly developed Ag, IgG and IgM recombinant ELISAs (rELISA) and improved capacity at the site, we are now able to accurately study viremia and the immune response. This proposal will test the *hypothesis*: **Development of IgM, but not a cellular inflammatory response, and decline in viremia during the early stages of symptomatic LF disease decreases mortality.** The *objective* of this proposal is to define the immune response to Lassa fever in symptomatic, hospitalized patients and to improve the clinical research capacity of this unique site. The long-term *goal* of this project is to further develop the highly productive partnership between Tulane University and the Sierra Leone Lassa Fever Project. This work is vital to the future design and implementation of definitive diagnostic and therapeutic trials.

Aim 1. To identify clinical and virological determinants of Lassa fever outcome in a cohort of symptomatic Lassa fever patients presenting to Kenema Government Hospital. Previous studies have identified several clinical features and clinical laboratory parameters that predict a poor prognosis. However, no study has determined if degree of viremia correlates with clinical features or laboratory test abnormalities. We will test the hypothesis: **Prolonged viremia correlates with disease progression and increased mortality.** We will identify clinical and laboratory diagnostic and prognostic risk factors for LF and correlate them with viremia and risk of mortality. This aim will establish the natural history of LF disease and determine if the time course can be divided into distinct stages as previously suggested.

A. We will correlate clinical characteristics of hospitalized patients with the quantitative Ag ELISA and standard laboratory tests, including WBC, Hgb, PT/INR, and metabolic panels, at enrollment and on days 2,3,4, 7 and 10 of their hospital stay.
B. We will compare multiple tools designed to predict hospital mortality, including modified SAPS II, APACHE II and APACHE IV, with a disease staging scheme and viremia.

Aim 2. To identify biomarkers of infection and mortality. Recent development of rLASV IgM and IgG ELISAs as well as increased capacity at the KGH Lassa Fever Laboratory provide a unique opportunity to identify specific immune correlates with disease severity and risk of mortality. We will test the hypothesis: **Delayed development of the humoral and an increased TH1 inflammatory immune response correlates with increased mortality.** We will correlate specific humoral and cytokine profiles with disease progression and outcome. These studies are critical for the design of future interventional trials and the development of therapies for LF and other viral hemorrhagic fevers.

A. We will correlate specific clinical characteristics and outcome with rLASV IgM and IgG ELISA and define their role as predictors of progression and outcome.
B. We will perform a pilot study to determine the feasibility of performing pro- and anti-inflammatory cytokine profiles at multiple time points during the hospitalization of symptomatic LF patients.

Aim 3. Clinical trial capacity training. Over the past decade, a significant amount has been learned about the pathogenesis of LF and the human immune response. Trials are now needed to test existing and novel therapies for this disease. The objective of this aim is to **build on previously funded capacity at the site and train critical team members in the essential elements needed to conduct clinical trials.** This training is critical if the KGH site is to continue to contribute to our knowledge of and management strategies for VHFs.

A. To build on the current clinical trial capacity, we will **establish a local hospital institutional review board (IRB)**.
B. To develop a mechanism for assessing fluid leakage in Lassa patients, we will **provide ultrasound training to KGH staff and implement ultrasound testing as a routine diagnostic**.
C. To establish the capacity for conducting an intervention-based clinical trial.

Research Strategy

Significance:

Lassa fever was first identified in Nigeria in 1969 (1). It is endemic to several countries in West Africa including Sierra Leone, Guinea, Liberia and Nigeria (Figure 1) (2-7). In addition, it has also been reported in Mali, Senegal and Ghana (8-10). Approximately 300,000 cases of LF occur per year causing 5,000 deaths (2). The natural reservoir for LASV is the multimammate rat (*Mastomys natalensis*) which is found throughout sub-Saharan Africa (11, 12). LASV is maintained in the rodents through vertical (mother to offspring) transmission (13). Humans are believed to acquire infection mainly via exposure to the excreta of infected rodents (2). However, there is compelling evidence that arenaviruses (LASV (14) and Junin virus (15)) are stable and infectious by the aerosol route in nonhuman primates. The aerosol route has also been occasionally suspected in human transmission (16-18), but infection via contact with abrasions in the skin, mucous membranes and ingestion are also viable routes of spread. Secondary transmission of LASV between humans occurs through direct contact with infected blood or bodily secretions. Nosocomial transmission and outbreaks have been described in healthcare facilities in endemic areas (19-22).

Several studies in the 1970's and '80's described the clinical presentation of this acute and frequently fatal hemorrhagic fever in very general terms. Approximately 80% of infections are believed to be asymptomatic or mild (23). After an incubation period of 1-2 weeks, symptomatic patients develop fever, headache, anorexia, dry cough and fatigue (1, 24, 25). Patients are frequently given an initial diagnosis of malaria or typhoid fever. Many other febrile illnesses are also included in the differential diagnosis (25). Over the next several days, patients may develop severe sore throat, retrosternal pain, tinnitus, conjunctival injection (red eyes), nausea, vomiting, diarrhea and abdominal pain (26, 27). A minority of patients progress and develop a capillary leak syndrome characterized by head and neck edema, bleeding, hypotension and shock (25). Obvious hemorrhage occurs in <20% of hospitalized cases and typically manifests as oozing from the mouth, nose, vagina, rectum and injection sites (6, 24). Laboratory investigation typically reveals mild thrombocytopenia, mild leucopenia and moderate hemoconcentration in hospitalized patients (24, 28-31). Elevated blood urea nitrogen (BUN) and proteinuria are also frequently seen as are increased amylase and hepatic transaminases. Aspartate aminotransferase (AST) is typically greater than alanine aminotransferase (ALT) and an AST >150 IU/L is considered a poor prognostic sign (24, 32, 33). No correlation between laboratory values and clinical features has ever been made. Reported case fatality rates for hospitalized cases range from 12% to 36% (27, 33, 34). Nigerian and nosocomial outbreaks have been associated with higher mortality rates than sporadic hospitalized cases. Rates are also higher for pregnant women (35). Women in the third trimester have mortality rates of up to 30% with fetal death rates approaching 100%. LF survivors have been noted to be at risk for several long-term sequelae including deafness in 25% (24, 36).

Available therapy for LF: No LF vaccine is currently available for use in humans. A 10 day course of intravenous ribavirin has been shown to decrease mortality of severe disease from 55% to 5% if given within the first six days of symptoms (37). Ribavirin is a Category X drug and has significant side effects including leukopenia, neutropenia, thrombocytopenia and hemolytic anemia. Thus, it has the potential to complicate patient management, especially in a population with a high incidence of anemia due to chronic malaria and sickle cell disease. At present, therapy of Argentine hemorrhagic fever, caused by Junin virus, a New World arenavirus, involves transfusion of immune plasma in defined doses of neutralizing antibodies during the prodromal phase of illness (38). Serum collected form LF survivors was initially used as therapy (39). However, studies have shown that pooled immunoglobulin containing high titers of anti-LASV antibodies is not effective (40-42). The efficacy of passive immunization for treatment of LF appears dependent on the titer of the neutralizing antibody infused. Geographic origin is also a factor as geographically matched plasma is more likely to contain adequate neutralizing titers against homologous Lassa virus strains.

Acquired immunity in LF. Bausch et al demonstrated that in patients with confirmed LF, LASV antigens indicative of viremia typically appear in the blood within the first week of illness (33). LASV-specific IgM appears in the second week of illness, and in most cases correlates with a rapid drop in the amount of viral antigens and nucleic acid. However, case reports have described the absence of any antibody response as well as robust responses in fatal cases (43). Low-titer neutralizing antibodies, if formed, typically appear several weeks to months after the resolution of infection (40, 42). Whether viral clearance results from the IgM response or as appears more likely from a combination of innate and adaptive humoral and cellular immune mechanisms is unknown. The presence of LASV-specific IgG suggests previous exposure to LASV. In addition,

it has been hypothesized that long-term sequelae, such as deafness may be due to the rapid appearance of IgG (36). The contribution of the proposed research is expected to be a greater understanding of the natural history of LF and the development of the immune response in symptomatic disease. *This proposal is significant because it is the first step in a continuum of research that is expected to lead to the development and testing of vaccines and pharmacologic therapies for LF. In addition, capacity building at the site will make future interventional trials possible.*

Why choose LF for human immunological study? Unlike many VHFs, LF is not a rare disease that emerges only as sporadic cases or outbreaks. Although surveillance is inadequate to determine the true incidence, up to 300,000 infections and 5,000 deaths from LF are estimated to occur yearly across West Africa (2, 23). The highest incidence of LF is in the "Mano River Union (MRU)" countries of Sierra Leone, Liberia, and Guinea (5, 6, 22, 44-47). Seroprevalence studies in eastern Sierra Leone suggest that 52% of the population in some areas have evidence of LASV exposure. LF is also the most frequently imported person-to-person communicable VHF (48). Ultimately, the high incidence of LF, along with the unique infrastructure and resources available in Sierra Leone offer one of the only opportunities in the world to conduct detailed and integrated studies of the correlates of protective immunity in a VHF and Category A Select Agent. *Such studies, as proposed here, are imperative to guide rational design of therapies, clinical management strategies and vaccines for VHFs*.

Innovation:

Pivotal studies performed in Sierra Leone by the Centers for Disease Control in the 1970's and 1980's characterized the epidemiology of LASV and identified important diagnostic and prognostic characteristics of severe LF (2, 24, 27). Several diagnostic modalities were used including virus isolation, immunofluorescent antibody (IFA) testing and IgM and IgG ELISAs utilizing cell lysates produced in BSL-4 laboratories (25, 27, 32, 33). Virus isolation, although extremely specific, and IFA pose several safety concerns, have limited sensitivity and are not practical for rapid diagnosis. BSL-4 reagents are also difficult and costly to produce and are in limited supply. Reverse transcriptase and real-time PCR are also used for diagnosis, but clean conditions and reagents required PCR make it a challenge for use in the field. Civil war in Sierra Leone forced the suspension of research activity and closure of the diagnostic laboratory in 1993. The Lassa Laboratory at Kenema Government Hospital (KGH), Sierra Leone reopened in 2005 and research resumed. *This site represents one of the few locations where cases Category A Select Agent Viral Hemorrhagic Fevers can be evaluated and treated on a routine basis.* Recently, new ELISA-based diagnostics utilizing recombinant proteins and monoclonal antibodies have been developed by the Viral Hemmorhagic Fever Consortium (www.vhfc.org) allowing for more cost-effective, reliable and safer testing of patient samples (49, 50). In addition, a LASV Antigen Rapid Test kit (Corgenix Medical Corp, Broomfield, CO) is also available (49). Both antigen detection platforms allow for quantitation of circulating antigen. *These new immunodiagnostics represent a significant improvement over diagnostics used in previous studies and will facilitate the further characterization of the natural history of LF and the humoral immune responses to LASV.* Finally, significant improvements in the infrastructure and capacity at KGH make analysis of the cellular and immune response to VHFs feasible. Our preliminary studies strongly suggest that this approach will redefine our understanding of the pathogenesis and improve our ability to detect and treat this deadly disease. This work is vital to the development of appropriate public health strategies to combat this deadly disease. In addition, it will provide the basis for further research on current and new therapies for VHFs.

Approach

Aim I: Identification of Clinical and Virological Determinants of Lassa Fever Outcome

Rationale: The time from illness onset to presentation is extremely difficult to determine in a country with extremely high illiteracy. The *objective* of this aim is to increase our understanding of the natural history of symptomatic LASV infection and to establish the utility of clinical markers for predicting disease stage and outcome. To attain this objective, we will test the *working hypothesis* that **prolonged viremia correlates with disease progression and increased mortality.** We will test our hypothesis by collecting clinical and laboratory data in a standardized fashion for all enrolled subjects. The *rationale* for this aim is that successful completion of the proposed research will contribute a missing, fundamental element to our base knowledge, without which studies of current and new therapeutic interventions cannot be fully interpreted. When the proposed studies of Aim #1 are completed, it is our expectation that clinical course and laboratory tests will

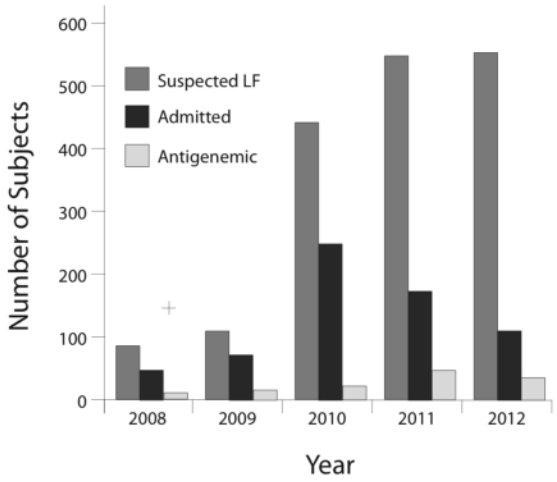

Figure 1. Evaluations per year for suspected LF at KGH (2008-2012). The number of suspected cases evaluated has increased steadily. Diagnostic IgM and Ag rELISAs were fully implemented in 2010. Mildly ill Ag-/IgM+ patients are currently referred to the general medical ward but will be admitted to the new, larger Lassa Fever Ward (See Facilities).

allow accurate diagnostic and prognostic risk assessments for LF patients. Such findings are of immense importance in the treatment of LF and the evaluation of new therapeutic approaches.

Justification and Feasibility: *Although a great deal was learned about LASV and LF, the early studies described above have several shortcomings. First*, early studies by McCormick et al described the clinical course of LF in hospitalized patients diagnosed on the basis of virus isolation or serology (24). However, the results of the diagnostic tests were not published. Therefore, any correlation between specific symptoms and any degree of viremia is not possible. *Second*, clinical laboratory studies were limited and results were not compared to controls in several studies (27, 32, 37). *Third*, clinical and laboratory studies were often only collected at presentation. Serial patient samples were generally not collected thus limiting our understanding of the course of LF disease. *Fourth*, previous studies have described, in general terms, the onset and durations of major signs and symptoms of LF (1, 24, 26). A staging system for LF severity has been presented in the popular press, but has never been validated in a clinical study (51). Severity scales allow for more accurate measurement of health status before, during and after a medical intervention (52). They can improve the design of clinical trials and allow for more precise evaluations of diagnostic efficiency and therapeutic effectiveness. Without a validated staging system, it is difficult, or impossible, to evaluate clinical outcomes and treatment efficacy of homogeneous patient clusters. These limitations make it difficult, if not impossible, to determine the natural progression of disease and hinder design and interpretation of future therapeutic trials.

Preliminary Studies: The discussion above illustrates that our understanding of the clinical progression of LF is incomplete. Our approach requires that we enroll patients who meet the definition of suspected LF and collect clinical and laboratory information at specified time points. The following preliminary data support the feasibility of this approach in our hands. Over 1,700 subjects with suspected LF were enrolled in ongoing NIH-sponsored Tulane research projects at KGH between 2008 and 2012 (Fig. 1). All subjects were tested for the presence of LASV Ag. 430 were admitted to the LFW based on clinical presentation or laboratory evidence of LF and 133 were antigenemic. As detailed below, a complete patient history and physical exam is recorded on standardized forms for each patient, including all signs and symptoms noted in previous studies. Facial edema, conjunctivitis, sore throat and bleeding on presentation were all strongly associated with a diagnosis of LF (Table 1). Increased INR was also associated with detection of LASV Ag, but not AST or creatinine. As shown in Table 2, mortality was highest in patients with LASV antigenemia on presentation as well as bleeding and a prolonged duration of illness. The LASV Ag ELISA has calibrators allowing for quantification of detected NP. In a recently completed study of the Ag ELISA performance, detection of ≥0.17 µg/ml was confirmed by PCR in 100% of cases and predicted mortality in 83% of cases. These data are important in establishing the feasibility of Specific Aim #1, because they demonstrate our ability to recruit subjects with both suspected and confirmed LF and our ability to carry out a complete clinical evaluations and laboratory testing, as well as quantify viremia, on a large patient cohort. They are merit further study, because previous studies have shown that elevated AST is a marker of mortality and coagulopathy does not occur in LF.

Table 1. Odds ratios for Lassa Fever Diagnosis

Characteristic (comparison)	Effective sample size	OR (95% CI)
Bleeding (yes vs. no)	1189	3.24 (2.19, 4.79)
Sore throat (yes vs. no)	1367	3.15 (2.25, 4.39)
Conjunctivitis (yes vs. no)	1364	3.04 (2.03, 4.56)
Head swelling (yes vs. no)	1181	3.27 (2.07, 5.16)
Duration of illness (≥7 vs. <7 days)	1273	3.09 (2.06, 4.64)
Cr (elevated vs. normal)	266	1.98 (0.84, 4.66)
AST (elevated vs. normal)	301	0.99 (0.22, 4.49)
INR (elevated vs. normal)	139	4.49 (1.40, 14.44)

Research Design:

Patient selection and enrollment. A standardized case-definition for suspected LF developed by staff at KGH and Tulane University is used to determine admission to the Lassa Ward, along with, as always, the attending clinician's judgment (Table 3). In general, 2 groups of patients will be enrolled for study: 1) patients with confirmed LF (see below), 2) patients with non-LF febrile illnesses. Patients with suspected LF will be enrolled directly from the Lassa Ward after written, informed consent is obtained.

Table 2. Selected odd ratios for mortality

Comparison	OR (95% CI)
Ag+ vs. Ag- patients	3.54* (1.82, 6.91)
Bleeding vs. non-bleeding symptoms	2.52* (1.24, 5.10)
Duration of illness ≥7 days vs. < 7 days^	1.95* (1.06, 3.59)

^ Defined as the interval between dates of illness onset and initial evaluation
* Statistically significant at the 5% significance level

Confirmation of LF cases and systematic sample collections and daily clinical observations. In this aim, we will define a confirmed, viremic LF case as one with a positive Ag ELISA and/or Ag rapid test and confirm this result by pcr. All samples will be run in duplicate. To detect changes in viremia over time, we will quantify viral antigen concentration by ELISA as illustrated in Preliminary Studies. Detailed daily clinical observations will be made on each patient confirmed to have LF from admission until discharge. Standardized and comprehensive data forms designed by Drs. Schieffelin, Grant and Khan are already in use. These forms prompt the physician to take a detailed history, including pertinent risk factors and length of illness, and to perform and record the results of a comprehensive physical exam, as well as routine vital signs, including oxygen saturation. They includes signs and symptoms relatively specific to VHF and LF, such as facial swelling, retrosternal pain, and hearing loss, that might not always be routinely sought. The timing of specimen collection will obviously be critical to interpretation of the laboratory findings. The treatment of LF calls for 10 days of intravenous ribavirin (37), so surviving patients are usually hospitalized for up to 2 weeks. Based on our previous experience in Kenema, and depending upon the patient's condition, we will be able to obtain blood from most patients at 5-6 time points during the course of their hospitalization for LF: at admission and on post-admission days 2, 3, 4, 7 and 10. Collection of a safe but sufficient volume of blood from acutely ill patients at each time point will also be critical to the success of this proposal. Although the volume that can safely be drawn will obviously vary by patient (due to factors such as size, clinical condition, hematocrit), based on our experience, 8-10 mls per blood draw is a reasonable estimate (about one tenth of a percent of a normal adult's blood volume). Due to biosafety concerns, all blood and body fluid specimens from suspected LF cases must be processed and tested in the Lassa Laboratory. Strict infection control and isolation practices are maintained on the ward and in the laboratory (53). A broad array of clinical laboratory tests will be performed using standard methods currently available in the KGH Lassa Laboratory (Table 4).

Table 3. Suspected Lassa fever case definition

- Known exposure to a person suspected to have Lassa fever OR
- Fever >38ºC for less than three weeks PLUS
- Absence of signs of local inflammation AND
- Two major signs or one major and two minor signs

Major Signs	Minor Signs
• Bleeding • Swollen neck or face • Conjunctivitis or sub-conjunctival hemorrhage • Spontaneous abortion • Petechial or hemorrhagic rash • New onset of tinnitus or altered hearing • Persistent hypotension • Absence of clinical response after 48hrs to anti-malarial and/or broad spectrum antibiotic therapy • Elevated liver transaminases, especially AST>ALT	• Headache • Sore throat • Vomiting • Diffuse abdominal pain/tenderness • Chest/retrosternal pain • Cough • Diarrhea • Generalized myalgia or arthralgia • Profuse weakness • Proteinuria • Leucopenia <4000/µl

Modified from Khan et al, 2008

Disease staging, severity analysis and predicted mortality. As discussed above, the time course and progression of symptomatic LF disease has described in general terms. However, no staging system has been tested or validated as a predictor of mortality. Based on the staging system shown in Table 5, the disease stage of each enrolled subject will be determined on a daily basis. Validation will require correlation with both changes in viremia over time and a published predictor of mortality. Since its publication in 1985, the APACHE II disease severity classification system has played a major role in determining the prognosis of severe disease as well as the efficacy of new therapies (54). This system requires several physiologic measurements not

available in resource poor settings such as Sierra Leone. Other groups have developed a Modified Apache II for use in areas such as KGH (55, 56). We have already adjusted our data collection forms and trained the KGH nurses to collect the required data including Glasgow Coma scores. We will calculate modified Apache II as well as other scoring systems such as the Apache IV and SAPS based on admission data. We will determine which system most accurately predicts mortality from acute LF. In addition, by collecting serial samples, we will correlate changes in viremia during ribavirin therapy with disease stage, severity score and in hospital mortality.

Table 4. Non-LF laboratory tests performed on all patients admitted to KGH Lassa Fever Ward

Admission Only	Admission and Hospital Days 2,3,4,7,10
Pregnancy test (females 15 to 50 years)	White Blood Cell Count and Differential
Rapid Malaria Test and Smear	Hematocrit
Rapid Dengue Test	Complete metabolic profile
Rapid Typhoid test	Prothrombin time and INR
Hepatitis B surface Ag	Urinalysis
HIV with patient approval*	

*testing, pre- and post-test counseling and treatment is available for free through the Sierra Leone National HIV Program, Ministry of Health and Sanitation

In years 3 through 5, we will augment our clinical assessment capacity and staging system with ultrasound (See Aim 3.2). Ultrasound has proven extremely useful in the staging of other VHFs such as dengue fever (57-59). Specifically, once training is complete for on site staff, we will routinely evaluate patients for intravascular volume status and vascular fluid leakage, specifically ascites and pleural fluid. This technique will greatly improve the management LF patients and make possible further studies in the management of VHFs.

Table 5. Lassa Fever Disease Staging

Stage	Signs and Symptoms
I	Fever, weakness, headache, myalgias
II	Pharyngitis, conjunctivitis, cough, abdominal pain, retrosternal pain
III	Facial edema, disorientation, respiratory distress
IV	Seizures, mucosal bleeding, coma

Data management, statistical analysis, and projected sample sizes. Efficient collection, management, and analysis of data will be essential to the scientific integrity of the proposed project. The datasets generated are projected to be large, and thus particular focus will be placed on data management techniques. Data will be collected using paper-based forms, which will be scanned and redacted of all personally identifiable information on a weekly basis. All key and secondary variables will be double entered into a database by separate users and compared to ensure their accuracy. Data analysis will be carried out using both parametric and nonparametric methods. For those cases where distributional assumptions are justified, parametric techniques such as logistic regression, linear regression, multilevel modeling will be used to carry out significance testing and provide their measures of association. Nonparametric techniques such as Fisher's Exact test, Wilcoxon signed rank test, and Spearman's rank correlation tests will be reserved for those cases where distributional assumptions are not justified or sample sizes are sparse. The number of study subjects is perhaps the most important factor for testing each of the hypotheses described in the specific aims. To this end, we conservatively anticipate enrolling 50 confirmed antigen positive LF cases per year (for a total of 250 cases over the course of the study), which is considerably more than could be reasonably enrolled for similar studies in other countries. This study requires 35 subjects to achieve 80% power to achieve its main objective to link viremia with morbidity and mortality outcomes.

Expected results and alternatives to be explored if caveats are encountered. We are confident that the dataset generated in this proposal will lead to important insights into the natural history of LF. The Ag ELISA and rapid test we have developed are extremely sensitive and specific. We expect to be able to acquire very reliable data on the level of viremia in both survivors and fatalities. We will also be able to definitively identify diagnostic and prognostic clinical and laboratory markers of severe LF and correlate them with trends in viremia during therapy. Overall, this aim will increase understanding of the natural history of symptomatic Lassa fever and to establish the utility of clinical markers and staging for predicting response to therapy and outcome. Such knowledge will increase our understanding of the pathogenesis of severe VHFs and allow for the appropriate development and testing of new therapeutic interventions.

Our working hypothesis is: prolonged viremia correlates with disease progression and increased mortality. Although our preliminary data (see *Justification and Feasibility*) solidly support this hypothesis, there is the remote possibility that it could be invalidated. One potential problem is the influence of ribavirin. Antigenemic LF patients are immediately started on ribavirin. This will almost certainly impact the observed degree of viremia, immune response and pathogenesis. In fact, it is possible that the side effects of ribavirin may negatively impact patient outcome. However, there is no ethical alternative. Nevertheless, a broad spectrum of pathology will certainly be noted, as patients present at various different stages of clinical development. A second potential problem is a mortality rate on the Lassa Ward that is higher than generally reported in the literature. The high severity of disease in previously enrolled patients could bias our study due to poor enrollment of mild cases. The current ward is small and outdated. Physicians frequently keep mildly symptomatic LF cases on the general medical ward if they do not feel ribavirin therapy is warranted. Thus, these patients were previously lost to our analysis. However, we have now instituted procedures to track and retest these subjects. In addition, with the opening of the new ward in late 2014, these patients can be comfortably admitted to the "low containment" wing.

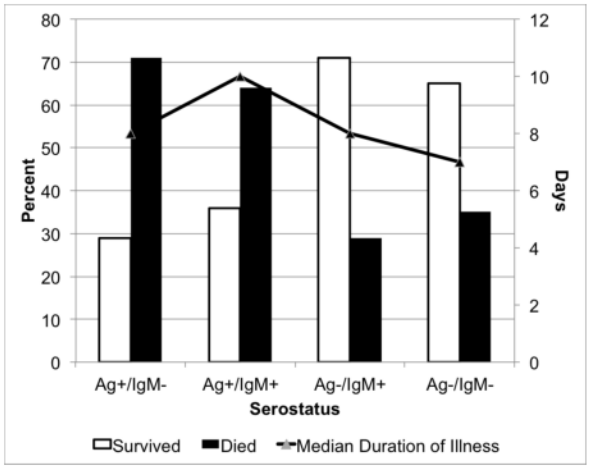

Figure 2. Mortality rate by serogroup. Patients presenting Ag+/IgM- had the highest mortality rate while Ag-/IgM+ patients had the lowest. No significant difference in mean time from illness onset to presentation was noted suggesting that some patients develop IgM faster than others.

Aim II: Identification of Biomarkers of Infection and Disease Severity

Rationale: Very little is known about the cellular immune response or the development of the IgM and IgG antibody response during acute LF. The *objective* of this aim is to identify specific serologic and cytokine markers that predict infection with Lassa virus and poor prognosis. To attain this objective, we will test the *working hypothesis* that delayed development of the humoral and an increased TH1 inflammatory immune response correlates with increased mortality and long-term sequelae. The *rationale* for this aim is that successful completion of the proposed research will contribute significantly to our understanding of the immune response to VHFs, without which new therapeutic interventions, such as immunotherapeutics and immunomodulators, cannot be designed. When the proposed studies of Aim #2 are completed, it is our expectation that the serologic and other laboratory tests will allow accurate diagnostic and prognostic risk assessments for LF patients. Such findings are of immense importance in the treatment of LF and the evaluation of new therapeutic approaches.

Justification and Feasibility: *Previous studies tested for humoral and cellular responses to LASV but were limited in several respects. First*, diagnostic techniques, though state of the art at the time, were insensitive and non-specific as well as dangerous. LF was confirmed in patients admitted to the hospital by viral isolation, four-fold increases in IFA titers or by elevated IgG and IgM titers at the time of admission (27, 32, 37). A later study demonstrated that Ag and IgM ELISA was a superior diagnostic platform for diagnosing acute LF (33). In addition, they showed that the presence of anti-LASV IgG is a marker of prior exposure and can be used to rule out acute LF. These results call into question the conclusions of the earlier studies. *Second*, IgM and IgG were rarely tested at multiple time points during hospitalization, making any correlation between outcome and the immune response impossible. *Third*, measurement of cytokines in the field was previously impossible due to the available capacity in LF endemic regions. Therefore, because of previous study design and in-country capacity, identification of LF diagnostic and prognostic specific markers has previously been impossible.

Preliminary Studies: Recent innovations in diagnostics and improved capacity at KGH have allowed us to conduct studies on the immune response to LF that were not previously possible. Our approach requires that we enroll patients who meet the definition of suspected LF and collect serologic and cytokine data at specified time points during and after hospitalization. We are fully confident that this approach is feasible as demonstrated by the following preliminary data. As discussed in Aim 1, over 1,700 subjects with suspected LF were enrolled between 2008 and 2012 and followed throughout hospitalization. All subjects were tested by ELISA at enrollment for circulating Lassa-specific IgM. 335 subjects had elevated Lassa-specific IgM

suggesting recent LASV infection. The overall mortality rate of suspected LF patients was 42/100. Antigenimic subjects with no evidence of an immune response on admission had the highest case fatality rate (CFR) at 73/100 (Fig 2). IgM+ subjects with and without antigenemia had lower CFRs of 55/100 and 28/100, respectively. These mortality rates are much higher than what has been reported in the literature. The mean time from onset of illness to presentation was similar for all four groups. These data suggest that, LF patients who have already produced LASV-specific IgM at the time of presentation are more likely to survive.

Our group has also done preliminary investigations into the cytokine profile of acutely infected patients (49). Blood collected at the time of presentation from suspected LF patients and serum levels of 25 different cytokines were measured by flow cytometry and compared to healthy controls. IL-6 and IL-10 levels were significantly elevated among fatal LF cases compared to nonfatal LF cases, non-LF febrile illnesses and healthy controls. IL-8 and MIP-1β were significantly lower in non-fatal vs fatal LF cases while IFNγ was significantly higher in fatal LF cases. These preliminary data suggest that low IL-6, IL-8, IL-10 and MIP-1β predict a positive outcome among LF cases. However, elevated IL-6 and IL-8 indicate a poor prognosis.

Recently, members of our team conducted a small pilot study on long-term morbidity and sequelae focusing on hearing loss. The KGH Outreach Team has extensive experience performing case investigations and performing post-discharge follow-up visits (see Resources and Facilities). In a recent study, we identified all patients given a diagnosis of LF between 2005 and 2012. We attempted to contact 356 subjects. We were able to find 55% of subjects or their immediate family members. Among those, 58% were deceased. This study demonstrates our ability to perform follow-up investigations. A sizable percentage of subjects were lost to follow-up, but this is not unexpected since many of these subjects had not been contacted for many years. In addition, it demonstrates that LF patients requiring hospitalization have a high mortality rate not only in the hospital, but also after discharge. Hearing tests were also conducted on a similar sampling of subjects. 40% of LF subjects had abnormal hearing while only 13% and 24% of non-LF febrile subjects and healthy controls had abnormal hearing, respectively. Collectively, these data demonstrate the feasibility and importance of contacting enrolled LF and control subjects after discharge and performing follow-up testing. As described below (see Research Design), in this proposal we will be following a much larger cohort than was tested in the preliminary studies. *Together, these data demonstrate the feasibility of objective of Specific Aim #2 and strongly support its working hypothesis that the delayed development of humoral and an increased TH1 inflammatory immune response correlates with increased mortality and long term sequelae.*

Research Design:

Patient selection and enrollment. Subjects will be enrolled as described in Aim 1. However, in this aim, 3 groups of patients will be enrolled for study: 1) patients with confirmed LF, 2) patients with non-LF febrile illnesses, and 3) afebrile household members and close contacts of LF cases. We will define a confirmed LF case with the rELISAs: 1. Positive Ag ELISA; 2. Four-fold rise in IgM titer from 2 specimens collected at least 48 hours apart or; 3. Seroconversion from IgM to IgG. As described above (see Innovation), we have developed sensitive NP antigen-capture and recombinant antigen-based IgG- and IgM-capture assays that can be used determine viremia and to adaptive humoral immunity to LASV in humans. Blood samples collected on admission and during hospitalization will be examined using these newly developed rELISA.

Seroconversion from IgM to IgG in convalescent LF patients. We will determine IgG and IgM titers to the LASV NP structural proteins using serum samples collected longitudinally on hospital days 1,2,3,4,7 and 10 of consented LF patients in the KGH Lassa Ward. Details of these diagnostics have already been published (49, 50). All samples will be run in duplicate. Internal quality control and standards will be included with each assay to allow for comparisons of sample run on different days. Like the LASV Ag ELISA, the IgM and IgG ELISAs are manufactured to commercial standards by Corgenix Medical Corp. (Broomfield, CO) providing rigorous QC/QA standards. Previous studies have shown that LASV-specific IgG appears about three weeks after infection (33). To capture this transition, we will need to collect samples from convalescent patients after discharge. We currently obtain blood during the convalescent period at 2, 8 and 24 weeks after illness, through follow-up visits by the KGH LF Outreach Team to the patient's home, which is standard practice both for public health as well as research purposes. The Outreach Team is also charged with case investigation and offering free testing to household members and close contacts of LF cases. Based on previous experience, collecting specimens in this manner often turns up mild or even asymptomatic cases. These cases will be important to the study, as they represent a "successful immune profile" in terms of controlling LASV infection.

Cytokine expression in acute LF cases. The preliminary data by our group presented above suggests that specific cytokine profiles can predict survival or death. Increased knowledge of the cellular immune response to LF can guide future therapeutic strategies. Again, we will use blood samples collected on days 1,2,3,4,7 and 10 of hospitalization from consented confirmed LF cases as well as febrile non-LF controls. Cases and controls will be sex and age matched in a 1:1 ratio. Peripheral blood mononuclear cells (PBMC) will be isolated on site by ficoll gradient centrifugation and utilized immediately. Because many samples will be highly infectious, it is not feasible to transport these samples back to Tulane University for analysis. To increase our understanding of the cytokine profile of LF survivors and non-survivors, we will use a two-stage approach. During year 1, in order to validate the findings presented in our preliminary results, we will test PBMCs collected from samples by intracellular cytokine staining (ICS) using the Accuri C6® benchtop cytometer (Accuri Cytometers Inc, Ann Arbor, MI) and the eBioscience FlowCytomix Human Th1/Th2 11-plex Kit (Bender MedSystems GmbH, Vienna Austria) as well as VEGF-A, CRP, RANTES, IFNα, and CD40L simplex kits. At the end of year 1, or after samples from 25 cases and 25 controls have been analyzed, we will perform an interim analysis. In years 2-5, we will focus only on specific cytokines with significant or potentially significant differences between cases and controls. We will continue to analyze PBMCs with ICS or, alternatively, use ELISPOT or ELISA.

Long-term mortality and morbidity. At each study visit during the follow-up period, we will determine each subject's overall health and collect blood for serologic analysis. A hearing test will also be performed using a standard audiometer. We will test each ear of each subject at three frequencies (500, 1000, 2000Hz) and decibel levels ranging from 25 dB (normal) to 75dB (profound hearing loss). We will then correlate the results of each of the above tests with progression of the immune response and determine if differences in frequency and severity occur between LF survivors and febrile controls. We expect that surviving cases will have a greater 6-month mortality rate compared with controls and that cases will have more memory loss and depression than controls. Finally, we expect to find a correlation between time to IgG appearance and frequency of hearing loss.

Statistical analysis, and projected sample sizes. The sample size assessment for Specific Aim 2 is based on the ability to detect a significant difference between the LF and NLFI groups with respect to their observed incidence of hearing loss. The methodology for the assessment is described by Schlesselman (60), and the calculations were performed using the PS software (61). The minimum required sample sizes are given below for varying incidence levels. The power and type I error are set at 80% and 5%, respectively. Previous pilot studies have shown that approximately 30% of LF cases and 15% of NLFI subjects experience hearing loss following hospital admission. Thus a sample of 133 LF patients and 133 NLFI patients will be required to show that the two groups are statistically different with respect to hearing loss at 80% power. Our proposed sample of 400 LF cases and considerably more NLFI subjects is more than adequate to meet the aforementioned criteria even with an expected mortality rate of close to 50%. The pilot data and sample size estimates are projected to be similar for comparing the groups with respect to incidence of depression following admission. Data will be analyzed using parametric techniques such as logistic regression, linear regression, multilevel modeling to carry out significance testing and provide their measures of association.

Expected results and alternatives to be explored if caveats are encountered. We fully expect to generate data that will lead to a greater understanding of the immune response and long-term effects of LF. The IgM- and IgG-capture ELISAs we have developed are extremely sensitive and specific. Multiple samples collected from each subject will allow us to identify immunologic risk factors for both long-term mortality and sequelae of LF. We expect to be able to acquire very reliable data on the time to appearance of IgM and ultimately seroconversion to IgG in both survivors and fatalities. We will also be able to definitively identify diagnostic and prognostic clinical and laboratory markers of severe LF. Overall, this aim will increase our understanding of the immune response during symptomatic LASV infection and to establish the utility of these markers for predicting disease severity and outcome.

Our working hypothesis is: the delayed development of the humoral and an increased TH1 inflammatory immune response correlates with increased mortality and long-term sequelae. Preliminary data by our group, as well as prior studies, strongly support this hypothesis. However, if our data could invalidate it, we would conclude that the maturity of the immune response to LASV has no impact long-term morbidity or mortality. It is also possible that some long-term sequelae and, even mortality, are due to the ribavirin treatment and not

LASV itself. To control for this, we will enroll household contacts with evidence of LASV infections as well as mildly symptomatic subjects in the hospital who do not require ribavirin therapy. These subjects, if asymptomatic, are not routinely treated with ribavirin at KGH. Therefore, they will serve as untreated controls.

Aim III: Clinical Trial Capacity Training

Rationale: For the past 10 years, a series of infrastructure improvements at KGH have been funded by the CDC, WHO, U.S. Department of Defense and NIH. These projects have resulted in a stable electrical system, a well equipped clinical and research BSL-3 laboratory and, most recently, a state of the art infectious disease ward specifically designed for the treatment of VHFs. Most members of the Sierra Leone National Lassa Fever Program have worked with LF for over 10 years. This highly experienced treatment and research team is at a critical juncture. In order for them to continue to contribute to LF research, a significant increase in their ability to conduct clinical trials is needed. The *objective* of this aim is to build on previously funded capacity at the site and train critical team members in the essential elements needed to conduct clinical trials. When this aim is completed, KGH and members of the research team will have all the essential elements and training in place to conduct clinical drug trials on LF and other infectious diseases.

Establishment of a local Institutional Review Board. To build on the current clinical trial capacity, we will **establish a local hospital institutional review board (IRB) at the Kenema Government Hospital (KGH)**. Significance: At a minimal cost, establishing a local IRB is a significant step toward initiating treatment-based clinical trials and expanding the research potential of the site. In turn, creating a local IRB will make the KGH more competitive for soliciting all types of research grants, including those without proposed interventions. A local IRB will also provide a local venue for researchers and external IRBs to seek advice. Indirectly, the effort will serve to foster local input from general community in performing research and provide education in an area where it is so badly needed.

Innovation: This aim seeks to develop the first local IRB at the world's epicenter for Lassa fever cases. The innovation lies in the developing an IRB in an area with severely limited resources that is endemic to Lassa fever.

Approach: The role of the proposed IRB is to provide local, focused IRB support at the KGH site. A lead physician at the KGH (Dr. Khan) and a board certified physician at Tulane (Dr. Schieffelin) will assume co-leadership roles for this effort. A draft set of policies and procedures will be established using federal guidelines (45 CFR 46 and 21 CFR 50 and 56) and adapted in accordance with local ordinances. The general IRB review process will include five phases: an application phase, a classification phase (i.e., full, expedited, or exempt review), a review phase, and a periodic review phase. Specific application forms will be developed including the following an application form, exempt, full review, expedited review, continued review, and adverse events. Checklist and decision tree forms will be developed for IRB personnel to ensure the review packet is complete. An organizational IRB structure will be developed and documented detailing each IRB member's qualifications and responsibilities. The KGH leader will initiate a series of meetings with other senior KGH personnel to determine potential personnel for filling each position. These persons will be interviewed and evaluated based on their qualifications, interest, and reliability. The committee will include at least five members, with at least one member representing each of the scientific, non-scientific, ethics, and local communities. A regular IRB meeting schedule will be established. One member will be designated as the committee's chair and another will be responsible for recording and archiving IRB minutes. A draft set of policies and procedures will be discussed at the first meeting. A set of case scenarios will be reviewed and used to develop the procedures. The final stage of IRB development will be to gain Federalwide Assurance (ref) approval. Upon completion, contact information for the new IRB will be made publicly available on relevant websites. All of the IRB minutes and procedures will be maintained electronically and reviewed and updated on an annual basis.

Three online training courses will be developed in the format the Collaborative Institutional Training Initiative (CITI). The courses consist of a series modules, short exams, and completion certificates. The first course will cover topics on overseeing human subjects research, reviewing protocols, protection of human subjects, securing personally identifiable data, record keeping, data monitoring, and IRB review protocol. The second course will focus on sustained, continued training and Good Clinical Practice (GCP) in research. The third training will cover critical concepts necessary to fulfill IRB-related responsibilities. The course materials will be maintained as part of the IRB's electronic archive. All IRB members will be required to complete and pass all three courses to become an official IRB member. All members will be required to complete the refresher

course every three years. New principal investigators at the KGH will also be required to take a subset of modules from these courses. The IRB will be responsible for ensuring that key research personnel have completed the training prior to beginning a study.

Training collaborators in ultrasound diagnostics: To develop a mechanism for assessing fluid leakage in Lassa patients, we will **provide ultrasound training to KGH staff and implement ultrasound testing as a routine diagnostic at the KGH**. Significance: The effort will help to uncover and characterize the underlying causes for a set of Lassa risk factors and will be used to guide therapy. We hypothesize that fluid leakage is among the chief contributors for Lassa-related fatalities and believe that detecting and monitoring fluid leakage will result in better treatment practices. Implementing ultrasound methods will also be particularly useful for pregnant patients, especially considering the high infant mortality rates and considerably higher infant mortality rates among Lassa cases.

Innovation: This specific aim establishes the first ultrasound diagnostic to quantify often subjective Lassa symptoms and lays the foundation for improving the quantitative capacity for diagnosing and treating Lassa fever patients. More broadly, the effort introduces new technology and customized local training in an environment with scarce resources. The data gathered from this effort will yield a more focused set of risk factors for more focused therapy.

Approach: Ultrasound machines will be reviewed in terms of their cost, portability, maintenance requirements, and a single machine will be purchased and transported to the KGH site (after pilot testing at the Tulane site). After completing a training course in clinical ultrasound in tropical infectious diseases, Tulane coordinator (Dr. Schieffelin) and a KGH physician (Dr. Khan or Dr. Grant) will lead the effort. The KGH coordinator will designate three KGH nurses and two KGH physicians to participate in the ultrasound testing initiative. A list of Lassa-related symptoms that may be quantified with ultrasound testing techniques will be developed and used to establish an ultrasound scanning protocol. The primary focus will be to measure Lassa-related symptoms involving fluid leakage (pleural fluid and ascites), intravascular volume status and pregnancy.

Scanning methods will be jointly evaluated and finalized by the KGH and Tulane coordinators. A set of standards will be developed for determining normal and abnormal scanning based on the first 50 patients scanned. A symptom-guided scanning protocol will be developed and used to monitor patient progress and response to therapy using comparative scans at different time points. For pregnant women, scans will be used to monitor the woman and the fetus while admitted to the KGH Lassa Ward. Use of an ultrasound logbook will be made part of standard protocol, and the all of the scans will be digitally archived and made part of each patient's electronic medical chart.

The Tulane coordinator will conduct on-site training to the KGH coordinator and relevant staff and oversee all of the initial scanning. A training course will be developed that includes specific modules for vascular, abdominal, ocular, and fetal scans. The course objectives will focus on basic sonography terminology and principles, ultrasound machine operation, using medical histories to guide the scans, imagery collection and archival, identifying patient abnormalities, and using the scan results in patient diagnosis. Each course will be approximately four hours in length, beginning with a presentation to introduce the fundamental ultrasound concepts and concluding with hands-on scanning exercises. All personnel responsible for scanning or using scans for patient diagnosis will be required to complete the training course. Upon completing the course, supervised training will be provided for performing scans on incoming suspected Lassa cases. A minimum number of scanning hours will be required to obtain official course completion and certification. A course manual will be developed and serve as a reference guide and starting point for continued development. More general training will offered via online continuing medical education (CME) courses.

Determine the capacity for conducting an intervention-based clinical trial: To bolster the expertise among KGH personnel for conducting an intervention-based clinical trial, we will facilitate and provide training to KGH clinical and data management personnel on the design and conduct of clinical trials.

Significance: This sub-aim will lay the foundation and develop expertise for conducting an intervention-based clinical trial according to U.S. standards in an area where death is rampant from Lassa fever and its current therapy is questionable. The objectives specifically focus on developing the capacity of local personnel and will contribute clinical research expertise where the number of clinical researcher is low and the need is great.

Innovation: We will train personnel for evaluating the effectiveness of ribavirin or testing a novel therapy for Lassa fever cases. The aim also establishes credentialed personnel where they currently do not exist and designates the site's first clinical research pharmacist. The aim takes a structured and standardized approach in a remote area where such training is commonly provided using unstructured, piecemeal mechanisms.

Approach: We will begin by carrying out a comprehensive clinical trial capacity assessment with senior KGH personnel. The assessment will prioritize the local infrastructure and training needs to comply with a U.S. FDA-sponsored clinical trial. We will request independent consultation by the International Clinical Studies Support Center (ICSSC, icssc.org) and combine the results of both assessments to form our official needs assessment. The assessment will be documented and serve as the baseline clinical trials capacity measurement. The assessments will be used to formulate a set of job descriptions and credentials to establish milestones and training goals. Key clinical trial personnel with will be identified according to their expertise in carrying out a hypothetical clinical trial. These personnel will be matched to a clinical trial position according to their expertise. The group will consist of physicians, clinical staff, data management personnel, and other relevant staff. Our next step will facilitate and provide training components to the clinical trial group to:

1. Understand the areas of bioethics and good clinical practice.
2. Develop data management plans, perform monitoring duties and report adverse events.
3. Meet regulatory requirements, document regulatory information, and maintain regulatory binders.
4. Understand the importance of data quality and management.
5. Implement flow-based process to ensure adherence to protocol steps.
6. Establishing a site clinical research pharmacist.
7. Demonstrate specific expertise credentials that make the site suitable for hosting intervention-based clinical trials.

To meet these objectives, we will focus pre-established training mechanisms through on-site training and self-guided learning tutorials. We propose using the ICSSC training mechanism with their titles and our objectives matched below (Table 6).

Table 6. ICSSC training assistance topics matched with proposed training objectives.

ICSSC training assistance topic	Format	Aim objective
Fundamentals of clinical research	On-site	1, 6, 7
Data management	On-site	2, 4, 7
Science writing	On-site	4, 6, 7
Biostatistics	On-site	4, 6, 7
Good clinical practice	On-site	3, 6, 7
Laboratory safety and quality	On-site, self-guided online training	3, 6, 7
Research ethics	On-site, self-guided online training	6, 7

Training will also include a customized module (developed by Tulane personnel) on how to use Consolidated Standards of Reporting Trials (CONSORT) and establish checklist-based approaches for carrying out clinical trials. We will seek on-site ICSSC training, but additional modules will be customized to local needs if their topics arise in the needs assessment. To lay the foundation for an on-site clinical research pharmacist, two current personnel will be selected as appropriate candidates according to their current job responsibilities. We will seek specialized training from the ICSSC to train the prospective clinical pharmacists on the use of patient medical histories to guide treatment therapy. A hospital room will be designated for treatment storage, and the prospective clinical research pharmacists will be responsible for overseeing the proper receipt and storage of all therapeutic drugs. Upon completing each of the training modules, credentials will be assigned in a digital log book and a certificate will be awarded to the trainee. Maintenance of the digital log book will be and incorporated into the on-site data manager's ordinary duties. In the unlikely event that on-site training cannot be provided by the ICSSC, we will facilitate the self-guided online training courses and the online training modules available through the Division of Microbiology and Infectious Diseases (DMID). The DMID tutorials cover topics on human subjects research (45 CFR Part 6), adverse and serious events, DMID regulatory file guidelines, essential regulatory documents, federal-wide assurance (FWA), study product accountability, and investigator responsibilities.

Project 2 Concept Proposal: Expansion of Clinical Research Capacity at Kenema Government Hospital

	Aim 1	Aim 2
Title	Identification of Clinical and Virological Determinants of Lassa Fever Outcome	Identification of Biomarkers of Lassa Fever Infection and Outcome
Hypothesis	Prolonged viremia correlates with disease progression and increased mortality	Delayed development of the humoral and TH1 pro-inflammatory immune response correlates with increased mortality
Objectives	Increase understanding of the natural history of symptomatic Lassa fever and to establish the utility of clinical markers for predicting disease stage and outcome	Identification of specific serological and cytokine markers that predict infection with Lassa virus and poor prognosis
Clinical Sites	Kenema Government Hospital	Kenema Government Hospital
Eligibility Criteria	Enrolled subjects must meet definition of suspected Lassa fever case	Enrolled subjects must meet definition of suspected Lassa fever case
Exclusion Criteria	1. Prisoners 2. Patients deemed too unstable on admission for any blood draws by treating physician	1. Prisoners 2. Patients deemed too unstable on admission for any blood draws by treating physician
Enrollment	2,500 suspected cases over 5 years, including 250 Antigen positive patients	2,500 suspected cases over 5 years, including 400 Antigen and/or IgM positive patients
Duration of Enrollment	10 days	6 months
Total Duration of Study	5 years	5 years
Endpoints/Outcomes	Death	1. Death 2. Hearing Loss
Summary of Data Analysis Plans	Repeated measures analysis modeling Lassa absorbance levels over time; survival analysis modeling time to survival outcome; frequency analysis comparing Lassa fever status and survival outcome	Repeated measures analysis modeling humoral and TH1 pro-inflammatory response rates over time; survival analysis modeling time to survival outcome; frequency analysis comparing Lassa fever status and survival outcome

This is the first submission by Tulane University and the Lassa Fever Program at Kenema Government Hospital to the International Collaborations in Infectious Diseases Research (U19) program.

A Progress Report Publication List is not applicable.

The human subjects research in Projects 1 and 2 meets the definition of "Clinical Research." Therefore, the research protocols and all consent/assent and data forms will be submitted to and approved by the Institutional Review Board (IRB) of Tulane and the Ethics Committees of Sierra Leone prior to implementation of the study. Informed consent will be obtained from all subjects prior to obtaining specimens for research. In some cases, execution of the study may entail testing anonymous sample remainders or serum specimens collected for other purposes. When appropriate, exemption from the requirement of IRB/Ethics Committee approval will be sought and obtained prior to testing the samples.

Patient identification and specimen collection. In Sierra Leone, blood is normally drawn immediately upon clinical suspicion of Lassa fever (which is most often at the time of admission) and then at various intervals thereafter, depending on the clinical course. Samples are collected in 10-ml syringes and stored on wet ice or cold packs and delivered to the KGH Lassa Laboratory. Samples are placed in the refrigerator and allowed to clot and the serum and cells then separated by trained technicians wearing standard personal protective equipment (i.e. masks, gowns, and gloves). Samples are labeled and stored at –20^{o}C until transport can be arranged to KGH (usually <48 hrs), where they are then sent by Ministry of Health-provided ground transport in specialized safety shippers on wet ice or cold packs.

Patient management and infection control. As Lassa fever is endemic in Sierra Leone, the hospitals and health posts in the region habitually see patients with the disease and have standard protocols for infection control. The study proposed here does not consist of a clinical trial and, in most cases, will not seek to obtain specimens from acutely ill patients that would not ordinarily be acquired through the clinically indicated management. However, to insure optimal management of the patient and the safety of the medical staff, with the consent of the hospital administration, patient isolation procedures and facilities will be reviewed and revised or up-graded as necessary. Training in infection control will be conducted and an adequate supply of personal protective equipment and other necessary supplies for the optimal care of patients with Lassa fever, such as syringes and intravenous fluids, will be supplied to each hospital. Although ribavirin is supplied for KGH and other hospitals in catchment areas for Lassa fever by the respective countries' Ministries of Health, supplies are often inadequate. Ribavirin is not approved by the U.S. Food and Drug Administration for the treatment of LF, and we will therefore not purchase or otherwise procure ribavirin. Procurement of ribavirin is at the sole the responsibility of the Sierra Leonean Ministries of Health. All decisions regarding patient care will rest with the local treating physician.

Risks to the subjects

Human subjects involvement and characteristics. Human subjects will be asked to provide blood, and occasionally other biological specimens, such as urine, for diagnostic testing and research. The subject population will be persons hospitalized at the Lassa Ward at KGH and partner institutions, who meet a specific case definition for Lassa fever. Blood specimens may also be obtained from patients during convalescence, as well as from family members or other members of their communities. There are no criteria for exclusion of any sub-population. Blood, blood cell components, serum, plasma, and other biological materials will be periodically sent from Sierra Leone to the other partner laboratories in the Project. All necessary permits will be obtained and biosafety regulations followed in the shipping of any biological specimens.

Sources of materials. Blood and other biological material will be obtained from the human subjects. All of the data obtained, including results of blood tests, will be recorded in both hard copy and electronic databases. Data and test results will be linked to the subject through their name, an assigned unique ID number, and other demographic information. Names will be used only when necessary and of benefit to the patient, such as communicating the patient's relevant clinical laboratory parameters to the treating physician. Only team members directly involved with the study will have access to subject identities. Specimens and data will be collected from the patients at the hospital. Many of the samples collected would normally be obtained for routine diagnostic purposes in the absence of this research project.

Potential risks. The potential risks to subjects in this study are thought to be minimal. The collection of blood may cause minor transient discomfort, but is rarely associated with any serious adverse effects. The amount of blood to be taken will not be enough to cause or exacerbate anemia. Subject names and all information taken will be kept confidential.

Adequacy of protection against risks

Recruitment and informed consent. The study protocol and all consent/assent and data forms will be submitted to and approved by the Institutional Review Boards and ethics committees of all the Project partner institutions/countries prior to implementation of the study. Informed consent will be sought from each patient. The healthcare worker/researcher will describe, in the local African language, the reason and overall plan for the study to all subjects, who will then be asked to participate. Informed oral consent will be recorded. Oral, rather than written,

consent is preferred due to the low literacy rate in the area involved in the study (~20%) and to the cultural hesitance about signing documents, which is a practice normally reserved for only the most serious transactions involving land or inheritance. Parental consent and subject assent will be obtained for persons under 16 years of age. At least two witnesses from the subject's family and two from the research team will be present for the informed consent. The subject's consent/assent and the witnesses' names will be recorded and kept on file.

Protection against risk. All blood drawing will be performed in a sterile fashion by trained personnel. Subjects' information will be kept strictly confidential. Hard-copy databases will be kept in a locked cabinet and electronic copies will be password-protected. A list of researchers who work with the databases will be kept at the laboratory and only those on the list will have access. A reminder of the need to maintain confidentiality will be given at weekly and monthly meetings. We believe these measures are likely to be effective. Although unlikely, subjects who acquire any problems, physical or psychological, related to the study will be instructed to consult with the doctor or nurse. Any charges related to patient care as a direct result of participation in the study will be incurred by the study.

Potential benefits of the proposed research to the subjects.
Participation in the study may afford a direct benefit to some of the subjects through knowledge of the results from the diagnostic and clinical laboratory assays that may help the treating physician better manage the patient's illness. Overall, the benefits to the subjects (independently of the long-term benefit of an increased understanding of the virology, immunology and natural history of Lassa fever seem to be reasonable, considering that the risks from participation in the study are minimal.

Importance of the knowledge to be gained
This study will result in a better understanding of the immunology and pathogenesis of Lassa fever. This will be important for developing countermeasures against these diseases around the world, whether through natural transmission or bioterrorism, including better diagnostic tests, therapies and vaccines.

Human Subjects research conducted in Projects 1 and 2 will involve approximately equal numbers of male and female subjects. Since the subject enrollment will take place in West Africa, 100% of the study population is expected to be black African. There are no exclusions.

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: Aim 1: Identification of Clinical and Virological Determinants of Lassa Fever Outcome

Domestic/Foreign: Foreign

Comments: Hypothesis: Prolonged viremia correlates with disease progression and increased mortality

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	1250	1250	0	0	**2500**
White	0	0	0	0	**0**
More than One Race	0	0	0	0	**0**
Total	**1250**	**1250**	**0**	**0**	**2500**

Study 1 of 2

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: Aim 2: Identification of Biomarkers of Lassa Fever Infection and Outcome

Domestic/Foreign: Foreign

Comments: Hypothesis: Delayed development of the humoral and TH1 pro-inflammatory immune response correlates with increased mortality

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	1250	1250	0	0	**2500**
White	0	0	0	0	**0**
More than One Race	0	0	0	0	**0**
Total	**1250**	**1250**	**0**	**0**	**2500**

Study 2 of 2

Funding Opportunity Number: RFA-AI-14-002. Received Date: 2014-03-07T14:26:44.000-05:00

Children of all ages will be included in the Human Subjects research conducted in Projects 1 and 2. The research team includes healthcare workers and facilities with extensive experience in dealing with children.

Project 2 does not use vertebrate animals.

Please refer to the Administrative Core for details regarding Select Agent Research.

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The Administrative Core will manage the Consortium Agreement between Tulane University and the Lassa Fever Program at Kenema Government Hospital.

Please refer to the Administrative Core for details regarding the Consortium Agreement.

All RFA required Letters of Support from Institutional and Government officials are compiled in the Administrative Core section of this application.

Please refer to the Administrative Core for details regarding Resource Sharing.

Grant Number: 5U19AI115589-02 REVISED
FAIN: U19AI115589

Principal Investigator(s):
Robert F Garry, PHD

Project Title: International Collaboration in Infectious Disease Research on Lassa fever

Tami Gomez Jenniskens
Tulane University of Louisiana
6823 St Charles Ave
New Orleans, LA 701122613

Award e-mailed to: elecnotf@tulane.edu

Period Of Performance:
Budget Period: 02/01/2016 – 01/31/2017
Project Period: 02/12/2015 – 01/31/2020

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to TULANE UNIVERSITY OF LOUISIANA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 31 USC 6305 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number U19AI115589. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Tina M. Carlisle
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5U19AI115589-02 REVISED

Award Calculation (U.S. Dollars)

Salaries and Wages	$110,116
Fringe Benefits	$23,774
Personnel Costs (Subtotal)	$133,890
Equipment	$16,728
Materials & Supplies	$174,636
Travel	$106,174
Other	$13,200
Subawards/Consortium/Contractual Costs	$267,419
Federal Direct Costs	$712,047
Federal F&A Costs	$216,090
Approved Budget	$928,137
Total Amount of Federal Funds Obligated (Federal Share)	$928,137
Less Unobligated Balance	$157,822
TOTAL FEDERAL AWARD AMOUNT	$770,315
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$0

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
2	$770,315	$770,315
3	$675,365	$675,365
4	$675,365	$675,365
5	$668,295	$668,295

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1720423889A1
Document Number:	UAI115589A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2016

IC	CAN	2016	2017	2018	2019
AI	8019650	$100,000			
AI	8472315	$670,315	$675,365	$675,365	$668,295

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M32B B / **OC**: 414P / **Released**: CARLISLET 01/18/2017
Award Processed: 01/18/2017 07:00:46 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5U19AI115589-02 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5U19AI115589-02 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.

b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

Carry over of an unobligated balance into the next budget period requires Grants Management Officer prior approval.

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) U19AI115589. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award is funded by the following list of institutes. Any papers published under the auspices of this award must cite the funding support of all institutes.

National Institute Of Allergy And Infectious Diseases (NIAID)

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal

Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5U19AI115589-02 REVISED

This award includes a carryover of **$157,822**(**$131,082** Direct Costs; **$26,740** F&A Costs) from the 01year to the **02** year. The carryover is approved for use only for the purpose stated in the request dated **11/23/2016** from Kathleen M. Kozar, Director Sponsored Projects Administration, Tulane University School of Medicine. The carryover will be subject to a downward adjustment should the available unobligated balance from the **02** year be less than **$157,822**; no adjustments will be made to the "TOTAL FEDERAL AWARD AMOUNT" on the Notice of Award.

Supersedes previous Notice of Award dated **06/21/2016**.
****

This award provides supplemental support for Ebola research for safety equipment to protect personnel. These funds **($66,445** Direct Costs and **$33,555** Facilities and Administrative Costs) are restricted for the purpose outlined in the application dated **04/04/2016** and may not be rebudgeted for other purposes. Funds awarded are available for carryover for awards given carryover authority as reflected in section III of this award notice. However, the funds remain restricted for the purpose for which the supplement is awarded.

A separate progress report is required under section G.1, Special Reporting Requirements, as part of the RPPR of the parent grant. The progress report for the supplement should describe what was accomplished under the supplement for the reporting period.

Supersedes Notice of Award issued 02/12/2016.
****

In addition to the PI, any absence, replacement, or substantial reduction in effort of the following individual(s) below, requires the written prior approval of the National Institutes of Health awarding component.

Dr. Jeffrey Schaffer, Co-PI, Project 1 Leader
Dr. John Schieffelin, Co-PI, Project 2 Leader
Dr. Donald Grant, Co-PI, Deputy Director of KGH Viral Hemorrhagic Fever Program
****

This award includes funds for subcontract/consortium activity with Kenema General Hospital in SIERRA LEONE and is budgeted as follows:

	-Yr 2	-Yr 3	-Yr 4	-Yr 5
Total Direct Costs	$252,899	$181,495	$181,495	$181495
F&A Costs @ 8%(MTDC)	$14,520	$14,520	$14,520	$14,520
TOTAL COSTS	$267,419	$196,015	$196,015	$196,015

Consortiums are to be established and administered as described in the NIH Grants Policy Statement. This written agreement with the consortium must address the negotiated arrangements for meeting the scientific, administrative, financial, and reporting requirements for this grant.
****

This award is issued as a Cooperative Agreement, a financial assistance mechanism in which substantial NIH scientific and/or programmatic involvement is anticipated in the performance of the activity. This award is subject to the Terms and Conditions of Award as set forth in Section VI: Award Administrative Information of RFA AI-14-001, "International Collaborations in Infectious Diseases Research (U01)," release date 11/27/2013, which are hereby incorporated by reference as special terms and conditions of this award.

This RFA may be accessed at: http://grants.nih.gov/grants/guide/index.html
****

Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at http://www.selectagents.gov/Regulations.html) must complete registration with CDC (or APHIS, depending on the agent) before using NIH funds for any work directly involving the Select Agent at the US institution. No funds can be used for research involving Select Agents if the final registration certificate is denied. Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS.

Before using NIH funds for any work directly involving the Select Agents at the foreign institution, the US awardee must provide information from the foreign institution satisfactory to the NIAID that processes and requirements comparable to those described in 42 CFR 73 for US institutions are in place and will be administered on behalf of all Select Agent work sponsored by NIH funds.

The US awardee must work with the foreign institution to ensure:

- That they understand the NIAID Select Agent Policy and requirements,
- That they are willing and able to allow the NIAID representative to enter and review the laboratories or facilities where Select Agent research is (or will be) conducted and the area(s) where NIAID funded select agents and toxins are stored,
- That they are willing and able to allow site reviews every three years after the initial review
- And that, during the visit, they are willing to address the following key elements appropriate for their institution: safety, security, incident response plan, training, procedures for personnel security risk assessment ensuring that only approved/appropriate individuals have access to the Select Agents, and any applicable laws, regulations and policies equivalent to 42 CFR 73.

If this work will not, in fact, involve Select Agents subject to the provisions of the US Select Agents regulations (e.g. excluded strains), and the US awardee provides documentation satisfactory to the NIAID that the work does not now nor will it in the future (i.e. throughout the life of the award) involve Select Agents at the foreign institution, no further action will be necessary.

Prior to conducting a restricted experiment with a Select Agent or Toxin at the foreign institution, the US awardees must request and receive approval from NIAID.
****

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Tina M. Carlisle
Email: carlislt@niaid.nih.gov **Phone**: 240-669-2947 **Fax**: 301-493-0597

Program Official: Patricia M. Repik
Email: prepik@niaid.nih.gov **Phone**: 240-627-3354 **Fax**: 301-480-1594

SPREADSHEET SUMMARY
GRANT NUMBER: 5U19AI115589-02 REVISED

INSTITUTION: TULANE UNIVERSITY OF LOUISIANA

Budget	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	$110,116	$103,751	$103,751	$103,751
Fringe Benefits	$23,774	$21,863	$21,863	$21,863
Personnel Costs (Subtotal)	$133,890	$125,614	$125,614	$125,614
Equipment	$16,728			$14,000
Materials & Supplies	$174,636	$106,191	$106,191	$94,191
Travel	$106,174	$71,500	$71,500	$71,500
Other	$13,200	$15,200	$15,200	$13,200
Subawards/Consortium/Contractual Costs	$267,419	$196,015	$196,015	$196,015
TOTAL FEDERAL DC	$712,047	$514,520	$514,520	$514,520
TOTAL FEDERAL F&A	$216,090	$160,845	$160,845	$153,775
TOTAL COST	$770,315	$675,365	$675,365	$668,295

Facilities and Administrative Costs	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	50.5%	50.5%	50.5%	50.5%
F&A Cost Base 1	$308,505	$318,505	$318,505	$304,505
F&A Costs 1	$155,795	$160,845	$160,845	$153,775
F&A Cost Rate 2	50.5%			

F&A Cost Base 2	$66,445			
F&A Costs 2	$33,555			
F&A Cost Rate 3	50.5%			
F&A Cost Base 3	$52,950			
F&A Costs 3	$26,740			

A. OVERALL COVER PAGE

Project Title: International Collaboration in Infectious Disease Research on Lassa fever	
Grant Number: 5U19AI115589-02	**Project/Grant Period:** 02/12/2015 - 01/31/2020
Reporting Period: 02/12/2015 - 01/31/2016	**Requested Budget Period:** 02/01/2016 - 01/31/2017
Report Term Frequency: Annual	**Date Submitted:** 12/02/2015
Program Director/Principal Investigator Information: ROBERT F GARRY , BS PHD **Phone number:** (504) 988-2027 **Email:** rfgarry@tulane.edu	**Recipient Organization:** TULANE UNIVERSITY OF LOUISIANA TULANE UNIVERSITY 6823 ST. CHARLES AVE NEW ORLEANS, LA 701185665 **DUNS:** 053785812 **EIN:** 1720423889A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: TAMI GOMEZ JENNISKENS TULANE UNIVERSITY OF LOUISIANA NEW ORLEANS, LA NEW ORLEANS, LA 70112 **Phone number:** 5045885613 **Email:** tjennis@tulane.edu	**Signing Official:** TAMI GOMEZ JENNISKENS TULANE UNIVERSITY OF LOUISIANA NEW ORLEANS, LA NEW ORLEANS, LA 70112 **Phone number:** 5045885613 **Email:** tjennis@tulane.edu
Human Subjects: Yes HS Exempt: No Exemption Number: Phase III Clinical Trial:	**Vertebrate Animals:** No
hESC: No	**Inventions/Patents:** No

B. OVERALL ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Lassa fever is an acute and often-fatal hemorrhagic disease caused by Lassa virus (LASV), an arenavirus. Lassa fever is a zoonotic infection, transmitted to humans by Mastomys natalensis (the multimammate “rat”). The highest incidence of Lassa fever in the world is in the Eastern Province of Sierra Leone. Signs and symptoms of LF, which occur 1-3 weeks after virus exposure, are highly variable, and can include fever, facial swelling, conjunctival injection, and vomiting. Frank bleeding occurs in less than a third of cases, but confers a poor prognosis. Signs and symptoms in LF patients who survive begin to subside 2-3 weeks after onset, but full recovery can require many months or longer.
Kenema Government Hospital (KGH) was an important site for Lassa fever clinical and laboratory research throughout the 1970s and 1980s. The violent civil conflict in Sierra Leone from 1991 to 2002, sometimes referred to as the Blood Diamonds War, forced suspension of Lassa fever research at KGH in 1993. Following cessation of hostilities, our consortium of Lassa fever researchers and other partners began rebuilding the scientific infrastructure at KGH, with a major focus of the development of improved laboratory diagnosis for Lassa fever. There is no vaccine for LF and the efficacy of the antiviral drug ribavirin in treating LF remains a matter of controversy. There is an urgent need to develop epidemiological and clinical measures to combat the public health challenges poised by Lassa fever in Sierra Leone and across West Africa. Promising next-generation Lassa fever diagnostic immunoassays, including rapid tests, require further development as epidemiological and clinical management tools.
Lassa ICIDR overall hypothesis: Further development of research capacity, with emphasis on training Sierra Leonean staff, will permit the KGH Lassa fever research program to emerge as an exceptional resource for human clinical trials of NIAID’s promising portfolio of Lassa fever diagnostics, therapeutics, immunotherapeutics and vaccines.
Overall Specific Aims (listed in priority order):
Aim 1. Further enhance and utilize the symmetrical and highly productive partnership developed over the last decade between Tulane University and the Lassa fever program at KGH.
Aim 2. Promote the development of laboratory and clinical research capacity at KGH, with a particular emphasis on training Sierra Leonean staff.
Aim 3. Encourage future collaborative relationships with other research groups leading to improvements in detection, prevention, amelioration, and treatment of Lassa fever in the subregion.
Research question(s) to be addressed in the Lassa ICIDR Projects include:
Project 1: Can second generation Lassa fever recombinant immunoassays be used effectively as point-ofcare diagnostics and surveillance tools for Lassa fever.
Project 2: What are the clinical and virological determinants of Lassa Fever outcome and is it possible to identify biomarkers of LASV infection and Lassa Fever outcome.
Through the proposed research we will acquire new information regarding the natural history of Lassa fever and the demographic distribution of people exposed to LASV using second generation Lassa fever recombinant immunoassays. We will also elucidate risk factors for acquiring serious or fatal LASV infection. This new information will guide evidence-based investments for public health programming and policy. We will also define biomarkers of Lassa fever pathobiology through the course of illness. Identification of these factors could lead to evidence-based approaches to reduce mortality from Lassa fever. We also propose to take a major step forward to

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Accomplishments ICIDR 11-15-15.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: training 11-15-15.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

The surveillance team continues their activities within the District in support of Projects 1 and 2. This is a concerted effort with the outreach team and the ecology staff respectively. We are actively recruiting subjects to identify clinical and laboratory diagnostic and prognostic risk factors for Lassa fever and will correlate them with viremia and risk of mortality. WE are also conducting training at PHS to improve diagnostic capacity for Lassa fever and Ebola at PHUs will enable community health workers to diagnose and refer suspected VHF cases earlier.

Weekly and monthly visits to communities and health facilities in search of Lassa will continue in Kenema, Kailahun, Pujehun and Tonkolili districts. During the reporting period, laboratory confirmed samples were investigated to determine their mode of contraction. According to surveillance investigations all of the Lassa cases reported contracted the disease from a primary source of infection, ie. the rodent Mastomys natalensis. Three cases died in the community. Community follow-ups will continue to be made in all the communities visited on the prevention and control of EVD and Lassa fever. Of the confirmed cases, we are seeing about 50% survival. Survivors will be visited in their respective communities three times in 2 weeks in every 3 months and 6 months to monitor any further complication. To monitor the progress of their health, samples will be collected for further laboratory investigation. All of these cases will be investigated at the KGH project laboratory.

Contacts tracing on Lassa fever and other VHF`s at district and community level will also continues based on laboratory confirmation of cases. All contacts will be monitored for three weeks (21days) period to monitor if any further case will develop or shows symptoms. This activity will continue alongside the Ebola contact tracing procedure checking for any health anomalies.

We will continue to build on previously funded capacity at the site and train critical team members in the essential elements needed to conduct clinical trials. As noted above a visit by FHI360, who provide consulting services for NIH, is scheduled for Dec 9 and 10 of 2015. WE expect that work will be completed in the first quarter of 2016 on the new viral hemorrhagic fever ward that will anchor the KGH ICIDR.

The Lassa and Ebola ICIDR has accomplished its year one goals of further development of research capacity, with emphasis on training Sierra Leonean staff. The KGH Lassa fever and Ebola research program is emerging as an exceptional resource for human clinical trials of NIAID's promising portfolio of Lassa fever and Ebola diagnostics, therapeutics, immunotherapeutics and vaccines.

The Lassa and Ebola ICIDR Administrative Core has carried out its mission to facilitate communication, planning, data sharing, and scientific and fiscal oversight of the component research projects of the ICIDR. The Core Program Managers have coordinated, scheduled, and implemented monthly internal meetings and teleconferencing among the Program sites, and assisted the Co-PDs of the Projects in the preparation of progress reports, internal compliance filings and editing of manuscripts to support the Program. The Core also oversaw data sharing, data archiving, and overall record keeping of the Program. The Administrative Core will be responsible for the overall organization, fiscal management and allocation of resources, decision-making, and evaluation of the Lassa and Ebola ICIDR. The Core sponsored its yearly symposium on VHFs in February 2015 at the Barmoi Hotel in Freetown, maintains a VHFC website (vhfc.org), and has supported relevant training of laboratory and clinical staff and other investigators as detailed in the next section.

Patients presenting with early signs of Lassa fever and Ebola are nearly impossible to distinguish from other febrile diseases, such as malaria and typhoid, based on clinical findings alone. Patients are more likely to visit a peripheral health unit (PHU) during the early stages of disease, when clinical signs and symptoms are nonspecific. These PHUs have limited laboratory capacity and personnel. Over 75% of patients presenting to the Kenema Government Hospital (KGH) Lassa Ward had symptoms for over 7 days. The delay in treatment likely attributes to the 69% case fatality rate in LASV antigenemic cases seen at the Lassa Ward. We have developed point-of-care and confirmatory diagnostics to commercial standards based on recombinant proteins. These immunoassays have high sensitivity/specificity for detecting infection with Lassa virus. We leveraged these advances to develop high sensitivity and specificity immunoassays for Ebola virus and other filoviruses, including rapid diagnostics that can be used in austere settings.

We are testing the hypothesis diagnostic capacity for Lassa fever and Ebola at PHUs will enable community health workers to diagnose and refer suspected VHF cases earlier. This will enable us to learn more about the early stages of these important diseases. Evaluate deployment of next-generation Lassa fever and Ebola recombinant antigen immunodiagnostics for point-of-care detection in peripheral health units is underway. We will establish feasibility of using reLASV and reEBOV diagnostics as an epidemiological tool for country-wide seroprevalence studies. We have begun studies to collect serum and utilize recombinant diagnostics to define correlates of immunity to Lassa fever and Ebola in patient contacts with little or no disease thereby identifying resistance patterns and susceptible cohorts for Lassa fever. We are also continuing to study Ebola survivors. We are building on our previous clinical study that showed that the West African variant of Ebola virus causes predominately a gastrointestinal illness and focused attention of this aspect of the disease for control of disease spread, rather than on bleeding manifestations that predominated in prior outbreaks.

There have been several accomplishments in Project 2, which seeks to expand clinical research capacity at Kenema Government Hospital. Using newly developed Ag, IgG and

IgM recombinant ELISAs (rELISA) and improved capacity at the site, we are now able to accurately study viremia and the immune response. We are actively recruiting subjects to identify clinical and laboratory diagnostic and prognostic risk factors for Lassa fever and will correlate them with viremia and risk of mortality. This aim will establish the natural history of LF disease and determine if the time course can be
divided into distinct stages as previously suggested.

We have also initiated studies to identify biomarkers of infection and mortality. We will test the hypothesis that delayed development of the humoral and an increased TH1 inflammatory immune response correlates with increased mortality. We will correlate specific humoral and cytokine profiles with disease progression and outcome. These studies are critical for the design of future interventional trials and the development of therapies for LF and other viral hemorrhagic fevers. We continue to build on previously funded capacity at the site and train critical team members in the essential elements needed to conduct clinical trials. The data collection and data management capacity at the Kenema Government Hospital (KGH) has also been improved, with Data manager Michael Gbacki having received training this year in North Carolina at an NIH sponsored workshop.

A visit by FHI360, who provide consulting services for NIH, is scheduled for Dec 9 and 10 of 2015. Work is being completed on a new viral hemorrhagic fever ward that will anchor the Khan Center of Excellence at KGH. The center is named after the late Dr. S. Humarr Khan who was the director of the KGH Lassa Ward and contracted Ebola treating patients in this outbreak.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

Three Kenema staff have been sent to the United State for training: Data Management training Michael Gbakie, Grant Management and Program Implementations: Ms. Simbirie C. Jalloh and Health research management: Dr. Donald S. Grant. These trainings are being supported and coordinated by the International Collaboration in Infectious Disease Research on Lassa fever (ICIDR). A laboratory staff Technician-Mambu Momoh recently attended a training session in the United States July this year. He was trained in Foundational Genomics hosted at Harvard University. Another training for Mr. Momoh was done to enable hjim to take responsibilities on Shippers/Cargo Agents on Transport of Dangerous Goods by air on Infectious substances. These will also include; Exempt Patient Specimens, Limited Expected Quantities, Genetically Modified Organisms, Dry Ice, Over Pack and Liquid Nitrogen. From this grants also, three other staff in Kenema Lassa fever program since January 2015 are been supported to continuing their studies at the Eastern Polytechnic. Upon completion, these staff will earn their Diplomas in Public Health and Laboratory Science.

C. OVERALL PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Safronetz D, Sogoba N, Lopez JE, Maiga O, Dahlstrom E, Zivcec M, Feldmann F, Haddock E, Fischer RJ, Anderson JM, Munster VJ, Branco L, Garry R, Porcella SF, Schwan TG, Feldmann H. Geographic distribution and genetic characterization of Lassa virus in sub-Saharan Mali. PLoS Negl Trop Dis. 2013;7(12):e2582. PubMed PMID: 24340119; PubMed Central PMCID: PMC3855028.
Complete	Matranga CB, Andersen KG, Winnicki S, Busby M, Gladden AD, Tewhey R, Stremlau M, Berlin A, Gire SK, England E, Moses LM, Mikkelsen TS, Odia I, Ehiane PE, Folarin O, Goba A, Kahn SH, Grant DS, Honko A, Hensley L, Happi C, Garry RF, Malboeuf CM, Birren BW, Gnirke A, Levin JZ, Sabeti PC. Enhanced methods for unbiased deep sequencing of Lassa and Ebola RNA viruses from clinical and biological samples. Genome Biol. 2014;15(11):519. PubMed PMID: 25403361; PubMed Central PMCID: PMC4262991.
Complete	Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, Levy DC, Yenni RE, Moses LM, Fullah M, Momoh M, Fonnie M, Fonnie R, Kanneh L, Koroma VJ, Kargbo K, Ottomassathien D, Muncy IJ, Jones AB, Illick MM, Kulakosky PC, Haislip AM, Bishop CM, Elliot DH, Brown BL, Zhu H, Hastie KM, Andersen KG, Gire SK, Tabrizi S, Tariyal R, Stremlau M, Matschiner A, Sampey DB, Spence JS, Cross RW, Geisbert JB, Folarin OA, Happi CT, Pitts KR, Geske FJ, Geisbert TW, Saphire EO, Robinson JE, Wilson RB, Sabeti PC, Henderson LA, Khan SH, Bausch DG, Branco LM, Garry RF, Viral Hemorrhagic Fever Consortium. Lassa fever in post-conflict sierra leone. PLoS Negl Trop Dis. 2014 Mar;8(3):e2748. PubMed PMID: 24651047; PubMed Central PMCID: PMC3961205.
Complete	Gire SK, Goba A, Andersen KG, Sealfon RS, Park DJ, Kanneh L, Jalloh S, Momoh M, Fullah M, Dudas G, Wohl S, Moses LM, Yozwiak NL, Winnicki S, Matranga CB, Malboeuf CM, Qu J, Gladden AD, Schaffner SF, Yang X, Jiang PP, Nekoui M, Colubri A, Coomber MR, Fonnie M, Moigboi A, Gbakie M, Kamara FK, Tucker V, Konuwa E, Saffa S, Sellu J, Jalloh AA, Kovoma A, Koninga J, Mustapha I, Kargbo K, Foday M, Yillah M, Kanneh F, Robert W, Massally JL, Chapman SB, Bochicchio J, Murphy C, Nusbaum C, Young S, Birren BW, Grant DS, Scheiffelin JS, Lander ES, Happi C, Gevao SM, Gnirke A, Rambaut A, Garry RF, Khan SH, Sabeti PC. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science. 2014 Sep 12;345(6202):1369-72. PubMed PMID: 25214632; PubMed Central PMCID: PMC4431643.
Complete	Kuhn JH, Andersen KG, Bào Y, Bavari S, Becker S, Bennett RS, Bergman NH, Blinkova O, Bradfute S, Brister JR, Bukreyev A, Chandran K, Chepurnov AA, Davey RA, Dietzgen RG, Doggett NA, Dolnik O, Dye JM, Enterlein S, Fenimore PW, Formenty P, Freiberg AN, Garry RF, Garza NL, Gire SK, Gonzalez JP, Griffiths A, Happi CT, Hensley LE, Herbert AS, Hevey MC, Hoenen T, Honko AN, Ignatyev GM, Jahrling PB, Johnson JC, Johnson KM, Kindrachuk J, Klenk HD, Kobinger G, Kochel TJ, Lackemeyer MG, Lackner DF, Leroy EM, Lever MS, Mühlberger E, Netesov SV, Olinger GG, Omilabu SA, Palacios G, Panchal RG, Park DJ, Patterson JL, Paweska JT, Peters CJ, Pettitt J, Pitt L, Radoshitzky SR, Ryabchikova EI, Saphire EO, Sabeti PC, Sealfon R, Shestopalov AM, Smither SJ, Sullivan NJ, Swanepoel R, Takada A, Towner JS, van der Groen G, Volchkov VE, Volchkova VA, Wahl-Jensen V, Warren TK, Warfield KL, Weidmann M, Nichol ST. Filovirus RefSeq entries: evaluation and selection of filovirus type variants, type sequences, and names. Viruses. 2014 Sep 26;6(9):3663-82. PubMed PMID: 25256396; PubMed Central PMCID: PMC4189044.

Complete	Kuhn JH, Andersen KG, Baize S, Bào Y, Bavari S, Berthet N, Blinkova O, Brister JR, Clawson AN, Fair J, Gabriel M, Garry RF, Gire SK, Goba A, Gonzalez JP, Günther S, Happi CT, Jahrling PB, Kapetshi J, Kobinger G, Kugelman JR, Leroy EM, Maganga GD, Mbala PK, Moses LM, Muyembe-Tamfum JJ, N'Faly M, Nichol ST, Omilabu SA, Palacios G, Park DJ, Paweska JT, Radoshitzky SR, Rossi CA, Sabeti PC, Schieffelin JS, Schoepp RJ, Sealfon R, Swanepoel R, Towner JS, Wada J, Wauquier N, Yozwiak NL, Formenty P. Nomenclature- and database-compatible names for the two Ebola virus variants that emerged in Guinea and the Democratic Republic of the Congo in 2014. Viruses. 2014 Nov 24;6(11):4760-99. PubMed PMID: 25421896; PubMed Central PMCID: PMC4246247.
Complete	Schieffelin JS, Shaffer JG, Goba A, Gbakie M, Gire SK, Colubri A, Sealfon RS, Kanneh L, Moigboi A, Momoh M, Fullah M, Moses LM, Brown BL, Andersen KG, Winnicki S, Schaffner SF, Park DJ, Yozwiak NL, Jiang PP, Kargbo D, Jalloh S, Fonnie M, Sinnah V, French I, Kovoma A, Kamara FK, Tucker V, Konuwa E, Sellu J, Mustapha I, Foday M, Yillah M, Kanneh F, Saffa S, Massally JL, Boisen ML, Branco LM, Vandi MA, Grant DS, Happi C, Gevao SM, Fletcher TE, Fowler RA, Bausch DG, Sabeti PC, Khan SH, Garry RF, KGH Lassa Fever Program, Viral Hemorrhagic Fever Consortium, WHO Clinical Response Team. Clinical illness and outcomes in patients with Ebola in Sierra Leone. N Engl J Med. 2014 Nov 27;371(22):2092-100. PubMed PMID: 25353969; PubMed Central PMCID: PMC4318555.
Complete	Lo Iacono G, Cunningham AA, Fichet-Calvet E, Garry RF, Grant DS, Khan SH, Leach M, Moses LM, Schieffelin JS, Shaffer JG, Webb CT, Wood JL. Using modelling to disentangle the relative contributions of zoonotic and anthroponotic transmission: the case of lassa fever. PLoS Negl Trop Dis. 2015 Jan;9(1):e3398. PubMed PMID: 25569707; PubMed Central PMCID: PMC4288732.
Complete	Boisen ML, Schieffelin JS, Goba A, Oottamasathien D, Jones AB, Shaffer JG, Hastie KM, Hartnett JN, Momoh M, Fullah M, Gabiki M, Safa S, Zandonatti M, Fusco M, Bornholdt Z, Abelson D, Gire SK, Andersen KG, Tariyal R, Stremlau M, Cross RW, Geisbert JB, Pitts KR, Geisbert TW, Kulakoski P, Wilson RB, Henderson L, Sabeti PC, Grant DS, Garry RF, Saphire EO, Branco LM, Khan SH, Viral Hemorrhagic Fever Consortium. Multiple circulating infections can mimic the early stages of viral hemorrhagic fevers and possible human exposure to filoviruses in Sierra Leone prior to the 2014 outbreak. Viral Immunol. 2015 Feb;28(1):19-31. PubMed PMID: 25531344; PubMed Central PMCID: PMC4287116.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. OVERALL PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	GRANT, DONALD SAMUEL	Personal Info	Personal Info		PD/PI	EFFORT			KGH	SIERRA LEONE	NA
	Y	Garry, Robert F			BS,PHD	PI						NA
	Y	Schieffelin, John Scribner			AB,MD,OTH	Co-Investigator						NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

No

E. OVERALL IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Dollar Amount	Country
200000	SIERRA LEONE

F. OVERALL CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. OVERALL SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Yes

Is the research exempt from Federal regulations?

No

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Report Attached: Aim 1: Evaluation of the deployment of next-generation Lassa fever recombinant antigen immunodiagnostics for point-of-care detection in peripheral health units.

Report Attached: Aim 3: To utilize ReLASV diagnostics to define correlates of immunity to Lassa fever in patient contacts with little or no disease thereby identifying resistance patterns and susceptible cohorts for Lassa fever.

Report Attached: Aim 1: Identification of Clinical and Virological Determinants of Lassa Fever Outcome

Report Attached: Aim 2: Identification of Biomarkers of Lassa Fever Infection and Outcome

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

No

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: Tulane University	053785812	LA-002	1430 Tulane Avenue New Orleans LA 701122699
Kenema Government Hospital	850510621	00-000	1 Combema Road Kenema
TULANE UNIVERSITY	053785812		TULANE UNIVERSITY 6823 ST. CHARLES AVE NEW ORLEANS LA 701185665
Tulane University	053785812	LA-002	1430 Tulane Avenue New Orleans LA 701122699
Kenema Government Hospital	850510621	00-000	1 Combema Road Kenema

G.9 FOREIGN COMPONENT

Organization Name: KGH
Country: SIERRA LEONE
Description of Foreign Component:
Kenema Government Hospital (KGH) in Sierra Leone, West Africa is a 350-bed facility situated in the heart of the region with the world's highest incidence of Lassa fever. Because of the importance of Lassa fever as a bioterrorism and public health threat, KGH has developed an advanced clinical and laboratory research capacity.

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Not Applicable

Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 1015468

Study Title: Aim 1: Evaluation of the deployment of next-generation Lassa fever recombinant antigen immunodiagnostics for point-of-care detection in peripheral health units.

Foreign/Domestic: Foreign

Planned Enrollment Report

Comments: Hypothesis: Deployment of point-of-care rapid diagnostics to peripheral health units (PHUs) will increase referrals to the LF Ward and reduce time to ribavirin treatment and mortality.

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	500	500	0	0	**1000**
White	0	0	0	0	**0**
More than One Race	0	0	0	0	**0**
Total	**500**	**500**	**0**	**0**	**1000**

Cumulative Enrollment Report

Only planned enrollment data exists for this data record. The PD/PI did not enter cumulative inclusion enrollment data.

Inclusion Data Record (IDR) #: 1015469

Study Title: Aim 3: To utilize ReLASV diagnostics to define correlates of immunity to Lassa fever in patient contacts with little or no disease thereby identifying resistance patterns and susceptible cohorts for Lassa fever.

Foreign/Domestic: Foreign

Planned Enrollment Report

Comments: Hypothesis: Active LASV+ case surveillance through contact tracing will identify early symptomatic and asymptomatic LASV-infected individuals. These individuals will have different biomarkers than those presenting at KGH through clinic referral and those without active LASV infection.

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	625	625	0	0	**1250**
White	0	0	0	0	**0**
More than One Race	0	0	0	0	**0**
Total	**625**	**625**	**0**	**0**	**1250**

Cumulative Enrollment Report

Only planned enrollment data exists for this data record. The PD/PI did not enter cumulative inclusion enrollment data.

Inclusion Data Record (IDR) #: 1015470

Study Title: Aim 1: Identification of Clinical and Virological Determinants of Lassa Fever Outcome

Foreign/Domestic: Foreign

Planned Enrollment Report

Comments: Hypothesis: Prolonged viremia correlates with disease progression and increased mortality

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	1250	1250	0	0	**2500**
White	0	0	0	0	**0**
More than One Race	0	0	0	0	**0**
Total	**1250**	**1250**	**0**	**0**	**2500**

Cumulative Enrollment Report

Only planned enrollment data exists for this data record. The PD/PI did not enter cumulative inclusion enrollment data.

Inclusion Data Record (IDR) #: 1015471

Study Title: Aim 2: Identification of Biomarkers of Lassa Fever Infection and Outcome

Foreign/Domestic: Foreign

Planned Enrollment Report

Comments: Hypothesis: Delayed development of the humoral and TH1 pro-inflammatory immune response correlates with increased mortality

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	1250	1250	0	0	**2500**
White	0	0	0	0	**0**
More than One Race	0	0	0	0	**0**
Total	**1250**	**1250**	**0**	**0**	**2500**

Cumulative Enrollment Report

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0	0	0	0	0	0	0	0	**0**
Asian	0	0	0	0	0	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	59	52	0	0	0	0	0	0	0	**111**
White	0	0	0	0	0	0	0	0	0	**0**
More than One Race	0	0	0	0	0	0	0	0	0	**0**
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	**0**
Total	**59**	**52**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**111**

RESEARCH & RELATED BUDGET - SECTION A & B

ORGANIZATIONAL DUNS*: 053785812

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY OF LOUISIANA

Start Date*: 02-01-2016 **End Date*:** 01-31-2017

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Robert	F	Garry		Project Lead	Institutional Base Salary	EFFORT			18,150.00	3,285.00	21,435.00
2.	Dr	Jeffrey	G	Shaffer		Investsigator					21,945.00	5,018.00	26,963.00
3.	Dr	Jessica	N	Hartnett		Investigator					24,385.00	6,267.00	30,652.00
4.	Dr	John	S	Schieffelin		Investigator					34,146.00	5,976.00	40,122.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **119,172.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Program Manager	EFFORT			5,125.00	1,317.00	6,442.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**6,442.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**125,614.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 053785812

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY OF LOUISIANA

Start Date*: 02-01-2016 **End Date*:** 01-31-2017

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
1. Motorcycle	10,000.00
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**10,000.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,000.00
2. Foreign Travel Costs	65,500.00
Total Travel Cost	**71,500.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		3,000.00
2. Stipends		0.00
3. Travel		5,000.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**8,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 053785812

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: TULANE UNIVERSITY OF LOUISIANA

Start Date*: 02-01-2016 **End Date*:** 01-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	98,191.00
2. Publication Costs	5,200.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**103,391.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**318,505.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	50.5	308,505.00	155,795.00
		Total Indirect Costs	**155,795.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**474,300.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: BUDGET JUSTIFICATION TULANE ICIDR YR2.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

ADMIN CORE
Senior/Key Personnel:
Robert F. Garry, Ph.D. will serve as Administrative Core Co-PD EFFORT months) and will be responsible for the day-to-day planning, initiation, implementation, conduct, monitoring, and completion of tasks identified Lassa ICIDR. Dr. Garry has managed NIH grants and contract (Cooperative Agreements) that successfully produced LASV recombinant antigen-based ELISA to LASV. Second generation LF Immunoassays are essential to achieving several Aims of the current Collaborative Program. He has demonstrated the ability to: coordinate, monitor, and manage multicomponent grant activities, including management of consortia to ensure project goals are met and timelines are kept; and effectively communicate with the project team and NIAID.

Christopher Bishop, MSW is Program Manager Assistant (PMA, EFFORT months) with excellent organizational and informational technology skills. He is in charge of all day-to-day administrative and logistical aspects of the project

Supplies:
Training materials and office supplies $623
Training is an important mission of the Administrative Core. Supplies ($500 per year) are requested to prepare training materials, which will be distributed to KGH investigators and staff. A budget ($123) for office supplies is also requested.

Domestic Travel: $2,000
As specified in the RFA, funds are requested for Dr. Garry to travel to the ICIDR Kick-Off Meeting within 3 months of award. In future years he will attend the Annual Programmatic Meeting.

International Travel: $4,500
Airfare to and from Sierra Leone costs approximately $2,500. In-country travel, hotel and per diem cost an additional $500 per trip. In addition excess baggage fees to transport materials to KGH average a total of $1500 per trip. We are budgeting for Dr. Garry to travel to the Sierra Leone site 1 time per year on Lassa ICIDR funds (total time for US Co-PIs in Sierra Leone exceeds RFA requirements for total US PI time spent at the International site). Dr. Garry regularly travels to Sierra Leone at least 4 times per year for a total stay of over 2 months.

Indirect Costs:
The Indirect Cost Rate for the Tulane University School of Medicine (TUHSC) is 50.5% (Modified Total Direct). Indirect costs are not applied to equipment or Consortium costs.

PROJECT 2
Senior/Key Personnel:
Jeffrey G. Shaffer, PhD, Co-Investigator, EFFORT is a Assistant Professor in the Department of Biostatistics and Bioinformatics at Tulane University School of Public Health and Tropical Medicine. Dr. Shaffer is an experienced statistician and data manager.

Jessica N. Hartnett, PhD, EFFORT is Director of Ecology and Community-based research for Tulane's Lassa Fever Program in the Department of Microbiology and Immunology. Dr. Hartnetts's has worked on Lassa fever in Sierra Leone since 2007 and is Tulane point-of-contact for KGH Outreach and Ecology teams. Dr. Hartnett will oversee Aims 1-3 of Project 1.

Supplies:

Lassa ELISA reagents are used for LASV screening and follow-up for all research subjects enrolled.
Point-of-care metabolic panel tests:. Lassa rapid test are used for distribution to peripheral health units for Project 1 Aim 1.

Research Lab reagents. laboratory consumables, sample storage, personal protective equipment. Desktops and tablets are used for KGH staff bioinformatics capacity building—computer training and data collection,

Equipment:
Two Honda XR motorcycles ($10,000 each) are requested for monitoring research activities in communities outside of Kenema Town. Costs in year 2 and 5 are for purchase of motorcycle, helmets, licensing, registration and insurance. Much of the community monitoring activities planned for the project can be performed by one individual, therefore transportation by motorbike ensures good value for money. Only experienced local personnel (non-Tulane staff) with motorcycle handler operator will operate motorcycles.

Foreign Travel: $25,000
Airfare to and from Sierra Leone is approximately $2,500 for each round-trip. Excess baggage costs for transporting of supplies and equipment costs approximately $1500 per trip with an additional $500 for incountry travel. Travel for four trips for Dr. Moses or Dr. Shaffer is planned for each year of the project.

Training
Funds are budgeted in each year for tuition and fees and other costs related to Training Programs, materials and travel costs for Sierra Leonean staff.

Indirect Costs:
The Indirect Cost Rate for the Tulane University School of Medicine (TUHSC) is 50.5% (Modified Total Direct). Indirect costs are not applied to equipment or Consortium costs.

PROJECT 2
Senior/Key Personnel:
John Schieffelin, MD, Project Co-Director EFFORT is Assistant Professor of Pediatrics and Internal Medicine at Tulane. He has been co-investigator on other Lassa fever projects funded by the NIH in Sierra Leone for over three years. He is also the clinical director of the Viral Hemorrhagic Fever Consortium, a partnership of research institutes whose mission is to promote global health and safety by creating new products to diagnose, treat and significantly reduce the incidence and mortality rate of viral hemorrhagic fevers. He has extensive experience managing clinical research projects at the study site, Kenema Government Hospital in Kenema, Sierra Leone. Dr. Schieffelin will be responsible with Dr. Donald Grant for the overall administration and direction of Project 2.

Jeffrey G. Shaffer, PhD, Co-Investigator (Data Manager), EFFORT is a Research Assistant Professor in the Department of Biostatistics and Bioinformatics at the Tulane University School of Public Health and Tropical Medicine. Dr. Shaffer is an experienced statistician and data manager and will coordinate the collection and statistical analysis of the data. He has worked with the Tulane University Lassa Fever Program for over 5 years. He maintains a database of all clinical and laboratory data collected at the Kenema site. He has significantly contributed to the study design and power and sample size calculations for proposed Project 2.

Supplies:
Blood collection supplies Lassa ELISA reagents (2nd Gen)
Significant costs are incurred during the care of Lassa fever patients. Although the Ministry of Health operates and maintains the Lassa Fever Ward at Kenema Government Hospital, additional supplies and staff support are necessary for clinical research. Vital supplies such as gloves, gowns, masks and other personal protective equipment are not reliably available in Sierra Leone. They are essential for protection of staff caring for patients suspected to have Lassa fever. Additionally, basic medical supplies are not always available in Sierra Leone such as thermometers, blood pressure cuffs, stethoscopes, etc. which are required for the clinical studies No funds will be used for basic clinical care or investigational drug purchases. Results with the second generation Lassa fever diagnostics will be compared to the first generation assays currently employed.

NOTE: All these supplies will be utilized at Kenema Government Hospital. Our practice is to purchase these supplies in New Orleans and then transport them to Kenema in boxes or trunks as excess baggage [each traveler can transport up to 9 "extra" pieces of luggage]. We also anticipate and have budgeted under the Administration Core for transport of one ocean-going container to KGH in each project year. The team is experienced in the logistics needed to maintain the laboratory and clinical operations at KGH. A limited number of supplies can be obtained locally and these are included under the consortium budget.

Publication Costs:
We anticipate that the year's activities will result in at least two peer-reviewed publication, which will be submitted to an open access journal. Publication fees for a typical open access journal such as PLoS Neglected Tropical Diseases are approximately $2600.

Domestic Travel: $4,000
As specified in the RFA, funds are requested for one of the Co-PIs (Dr. Schieffelin or Dr. Shaffer) to travel to the ICIDR Kick-Off Meeting within 3 months of award. In future years one of the Co-PIs will attend the Annual Programmatic Meeting.

International Travel: $36,000
Airfare to and from Sierra Leone costs approximately $2,500. In-country travel, hotel and per diem cost an additional $500 per trip. We are budgeting for Drs. Schieffelin and Shaffer, who will also serve with Dr. Garry as overall Lassa ICIDR Co-PIs, to each oversee research activity at the Sierra Leone site 4 times per year (total time for US Co-PIs in Sierra Leone exceeds RFA requirements for total US PI time spent at the International site). In addition, as discussed under Specific Aim 4, KGH personnel will travel to Tulane University or other locations in the United States for short periods of intense training in laboratory techniques, ultrasound training bioethics, regulatory requirements and data management. Indirect Costs:

The Indirect Cost Rate for the Tulane University School of Medicine (TUHSC) is 50.5% (Modified Total Direct).Indirect costs are not applied to equipment or Consortium costs.

RESEARCH & RELATED BUDGET - SECTION A & B

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 02-01-2016 **End Date*:** 01-31-2017

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Donald	S	Grant		Project Lead	Institutional Base Salary	EFFORT			23,500.00	3,525.00	27,025.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **27,025.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
15	PM, Nurses, Lab, Support, etc	EFFORT			62,000.00	7,800.00	69,800.00
15	**Total Number Other Personnel**					**Total Other Personnel**	**69,800.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**96,825.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 02-01-2016 **End Date*:** 01-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	26,000.00
2. Foreign Travel Costs	1,500.00
Total Travel Cost	**27,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		9,000.00
2. Stipends		5,000.00
3. Travel		1,000.00
4. Subsistence		505.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**15,505.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 8505106210000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Kenema Government Hospital

Start Date*: 02-01-2016 **End Date*:** 01-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	56,185.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**56,185.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**196,015.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**196,015.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: BUD JUST KGH ICIDR Year 2.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Senior/Key Personnel:
Donald S. Grant, M.B.Ch.B., will serve as Administrative Core Co-PD EFFORT months) and will be responsible for the day-to-day planning, initiation, implementation, conduct, monitoring, and completion of tasks identified Lassa ICIDR. Dr. Grant will also serve as overall Co-PI of the Lassa ICIDR. He is Director of the National Lassa Fever Program of the Ministry of Health and Sanitation (MOHS) in Sierra Leone. He is permanently assigned to Kenema Government Hospital. Dr. Grant is responsible for patient admission, testing and medical care on the Lassa Fever Ward. As Director of the National Lassa Fever Program, he also oversees activities of the Lassa Fever Laboratory and Outreach Teams.

Simbirie Jalloh will serve as Program Coordinator (PMA, EFFORT months). Ms. Jalloh has 5.5 years experience and excellent organizational and informational technology skills. She will be responsible for all dayto- day administrative and logistical aspects of the managing the project in Sierra Leone. Ms. Jalloh has visited the US twice, meeting with NIH program officers, contracting officers and other budgetary officials. She has received training from and works closely with investigators, program managers and grants and Contracts personnel at Tulane University and other subcontractors. Ms. Jalloh will manage the budget, keep financial and other records. She will arrange transport and accommodation for visiting scientists.

Supplies:
Training materials and office supplies $875
Training is an important mission of the Administrative Core. Supplies ($375 per year) are requested to prepare training materials, which will be distributed to KGH investigators and staff. A budget ($500) for general office supplies is also requested.

Travel from Sierra Leone to the United States): $4,000
Airfare to and from Sierra Leone costs approximately $2,500. In-country travel, hotel and per diem cost an additional $500 per trip. As specified in the RFA, funds are requested for Dr. Khan to travel to the ICIDR Kick- Off Meeting within 3 months of award. In future years he will attend the Annual Programmatic Meeting.
Travel within Sierra Leone: $1,500

Dr. Grant will visit each of the field sites at least once per year. He also will meet regularly with the central MOHS to inform them of Lassa ICIDR progress and results.
Indirect Costs:
None requested

PROJECTS 1 amd 2
Senior/Key Personnel:
Donald S. Grant, M.B.Ch.B, will serve as the Co-Director of Project 1 EFFORT and will be responsible for the overall supervision and coordination of all activities of the Project. Dr. Grant has extensive experience overseeing and managing research projects in Sierra Leone. Trained in Freetown, Sierra Leone Dr. Grant is Chief Clinician in the Lassa Ward, and is currently also a lecturer in the Community Health Department of the College of Medicine and Allied Health Sciences at the University of Sierra Leone. Along with his clinical work in the Lassa ward, Dr. Grant has been an investigator for a wide range of NIH-sponsored research studies. As an MOHS officer, his mandate is to build capacity, provide technical support, and coordinate regional efforts to confront LF and

other communicable diseases in Sierra Leone, Liberia, and Guinea.

Laboratory:
Augustine J. Goba, Senior Laboratory Technician, Director of the Lassa Fever Laboratory. Mr. Goba has over 25 years of experience as a Senior Laboratory Technician having served as Chief Technician on the Centers for Disease Control and Prevention (CDC) Lassa Fever Project in Kenema, Sierra Leone from 1987 to 1994. During the Civil War in Sierra Leone, Mr. Goba worked as a Chief Laboratory Technician in the CDC Guinea Lassa Fever Project, N.'Zérékoré, Guinea before returning to Kenema after the War in 2005 to resume his position at the KGH Laboratory. Mr. Goba will be primarily responsible for performing the Lassa virus ELISAs and oversees routine hematology and chemistry testing of samples in the Lassa Fever Laboratory. He also supervises two medical technologists each with over five years experience, Mohamed Fullah and Mambu Momoh.

Nurses:
Ms. Veronica Koroma, **assistant nursing supervisor** and midwife, has over 15 years experience treating Lassa fever patients. She has devoted much of her life to the treatment and research of Lassa fever. Ms. Koroma is responsible for ensuring that nursing coverage is adequate and oversees the care of all acutely ill patients on a day-to-day basis.

Mr. Michael Gbakie is a registered nurse in Sierra Leone and serves as the Clinical Research Coordinator for the Tulane Lassa Fever Program. He has over 10 years experience with management and research of Lassa fever. Mr. Gbakie is responsible for the quality control of the onsite research. In this position, he is for ensures that all enrolled subjects are consented properly and that all case report forms are completed appropriately. In addition, he supervises a laboratory technician.

Support Staff:
Victor Lungay will serve Database Manager/Statistician and will oversee data entry, statistical analysis, and interpretation as he does currently for WHO-sponsored projects at KGH. Two part-time cleaners work in the Lassa Laboratory and Lassa Laboratory.

Drivers:
Drivers, mechanics (M Sow, M Fomgbeh 50%) will support the Outreach and Ecology teams.

Outreach Team:
Lansana Kanneh a Sierra Leonean has 7 years experience in research projects with Tulane University and 20 years of experience on LF community education and surveillance. He is fluent in Mende and Krio and is extremely knowledgeable about the distribution of Lassa fever cases and geography of Sierra Leone. Mr. Kanneh, as supervisor of the Lassa Fever Outreach Team oversees case investigations and contact tracing of all Lassa fever cases diagnosed at IGH. In addition, he oversees and coordinates follow-up sample collections from Lassa patients discharged from the Lassa Ward. Mr. Kanneh will supervises a team of thee Outreach
Team members.

Ecology Team:
Ecology Team leader Mr. James Koniga will supervise the ecology team and perform

rodent trapping. Supervisor Koninga, has participated in collection of small mammals for zoonotic disease research since 1979 when he was trained by Centers for Disease Control and Prevention personnel during the first documented community epidemic of Lassa fever.Mr. Kargbo will be assisted by Kandeh Kargbo and Willie Robert.

Supplies:
Additional supplies and staff support are necessary for clinical research. Extra personnel are required for clinical research at this site including nurses, study coordinators, ambulance drivers and outreach workers for case investigation and patient follow-up visits. No funds will be used for routine clinical support or investigational drug purchases. Materials and supplies are needed for production of data collection forms, electronic tablets for data collection, GPS units, field team supplies.

Domestic Travel:
PRPJECTS 1 and 2 require follow-up visits to locations outside of Kenema Government Hospital each year, requiring logistic support and fuel costs. Several of the regions are a day's drive from Kenema Government Hospital. Housing costs will be re-imbursed will be given to members of
the Lassa Fever Program Outreach Team who must spend several days in the field for these visits.

Training:
Funds are budgeted in each year for tuition and fees and other costs related to Training Programs, materials and travel costs for Sierra Leonean staff that will be administered in Sierra Leone institutions, including the University of Sierra Leone (Freetown) and Eastern Polytechnic Institute (Kenema).

Other Expenses:
Town power in Kenema is highly unreliable. Town power is available about 5% of the time in Kenema, and even during those periods frequent intermittent or sustained power outages occur. Although the KGH Lassa Laboratory has limited solar power capability it is not sufficient to supply the power needs of the Lassa Ward, which relies on a generator. $8000 per year is requested for diesel fuel.
Funds are requested for maintaining the high-speed Internet connectivity amongst the Lassa ICIDR units ($6000 per year) and for upgrading software, computers and maintaining data management tools ($3,537 per year).

Indirect Costs:
None requested.

CONFERENCE GRANT **Issue Date:** 08/20/2013
Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 1R13AI104216-01

Principal Investigator(s):
Robert F Garry, PHD

Project Title: Opening a New Lassa Fever Ward in Kenema Sierra Leone

Ms. Kozar, Kathleen M
Director
1430 Tulane Avenue, EP-15
New Orleans, LA 701122632

Award e-mailed to: elecnotf@tulane.edu

Budget Period: 09/01/2013 – 08/31/2014
Project Period: 09/01/2013 – 08/31/2014

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $8,000 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to TULANE UNIVERSITY OF LOUISIANA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R13AI104216. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F. Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation; until then, Awardees must comply with the 1995 regulation. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Tina M. Carlisle
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 1R13AI104216-01

Award Calculation (U.S. Dollars)

Federal Direct Costs	$8,000
Approved Budget	$8,000
Federal Share	$8,000
TOTAL FEDERAL AWARD AMOUNT	$8,000
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$8,000

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$8,000	$8,000

Fiscal Information:

CFDA Number:	93.855
EIN:	1720423889A1
Document Number:	RAI104216A
Fiscal Year:	2013

IC	*CAN*	*2013*
AI	8472387	$8,000

NIH Administrative Data:
PCC: M74B B / **OC**: 415A / **Released**: eRA Commons User Name 08/14/2013
Award Processed: 08/20/2013 12:11:50 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R13AI104216-01

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R13AI104216-01

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Direct charges for meals/food and beverages are unallowable charges to this project.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/#gps .

A final Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 90 days of the expiration date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) cash transaction data.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 90 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm.

Unless an application for competitive renewal is submitted, a final progress report must also be submitted within 90 days of the expiration date. Instructions for preparing a Final Progress Report are at: http://grants.nih.gov/grants/funding/finalprogressreport.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the final progress report. Institute/Centers may accept the progress report contained in competitive renewal (type 2) in lieu of a separate final progress report. Contact the awarding IC for IC-specific policy regarding acceptance of a progress report contained in a competitive renewal application in lieu of a separate final progress report.

NIH **strongly encourages** electronic submission of the final progress report and the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final progress report and final invention statement may be e-mailed as PDF attachments to the NIH Central Closeout Center at: DeasCentralized@od.nih.gov.

Hard copy: Paper submissions of the final progress report and the final invention statement may be faxed to the NIH Division of Central Grants Processing at 301-480-2304, or mailed to:

NIH Division of Central Grants Processing, OER
6705 Rockledge Drive
Suite 5016, Room 5109
MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express mail delivery only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final Progress Report is not required. However, a final FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

Treatment of Program Income:
Additional Costs

Direct charges for meals/food and beverages are unallowable charges to this project.
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PUBLICATION REQUIREMENTS AND FINAL PROGRESS REPORT REQUIREMENTS - ADDITIONAL INFORMATION

All conference material (promotional materials, agenda, publications an internet sites) related to this project must include an acknowledgement of NIH grant support and a disclaimer stating the following: "Funding for this conference was made possible [in part] by (insert grant number) from the National Institute of Allergy and Infectious Diseases. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government."

Less than first class accommodations on U.S. flag carriers must be used (1) for departure from and entry into the U.S., and (2) for any other portions of the trip where U.S. carriers are available. The organization receiving an award assumes legal and financial responsibility and accountability both for the awarded funds and also for the performance of the supported activity. The awarding unit does not intend to restrict grantee organizations to any set plan in developing meetings.

In addition to the final progress report guidance provided in Section III above, please include the following in the final progress report:

- Grant number
- Title, date and place of the meeting
- Name of the person shown on the application as the conference director, principal investigator,or program director
- Name of the organization that conducted the meeting
- List of the individuals who participated as speakers or discussants in the formally planned sessions of the meeting and their institutional affiliations

Copies of proceedings or publications resulting from the meeting, including the items listed above, may be submitted for the final progress report, with approval of NIAID.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Tina M. Carlisle
Email: carlislt@niaid.nih.gov **Phone**: 301-402-6579 **Fax**: 301-493-0597

Program Official: Barbara L. Mulach
Email: bmulach@niaid.nih.gov **Phone**: 301-402-4199 **Fax**: 301-480-4528

SPREADSHEET SUMMARY
GRANT NUMBER: 1R13AI104216-01

INSTITUTION: TULANE UNIVERSITY OF LOUISIANA

Budget	***Year 1***
TOTAL FEDERAL DC	$8,000
TOTAL FEDERAL F&A	
TOTAL COST	$8,000

Final Progress Report
1R13AI104216-01
Opening a New Lassa Fever Ward in Kenema Sierra Leone
Tulane University
09/01/2013 to 08/31/2015
Robert F. Garry, PhD

Summary of progress made toward the achievement of the originally stated aims and significant results (positive or negative).

Specific Aims:
1. Commemorate the opening of the new Lassa Ward, reflect on the historical, societal and scientific impact of LF, and increase public awareness of the continued impact of LF in Sierra Leone and the West African subregion.
This aim was achieved. A day capacity building training for laboratory and diagnosis on Lassa and Viral Hemorrhagic fever was held in Sierra Leone on the 23rd July 2015.

2. Assemble several generations of experts on LF who may otherwise not have an opportunity to meet to share and debate their latest findings and to define goals for the future.
This aim was achieved. Speakers included the Deputy Minister of Ministry of Health Sanitation, Director of Hospital and Laboratory Services, Professor Robert Garry and many others.

3. Present training exercises and workshops to showcase new capabilities in West Africa for LF clinical and laboratory research.
This aim was achieved. Dr. Donald S. Grant, Director of the LF Program at KGH, presented an overview of operations at KGH with an emphasis on clinical management of Lassa fever. Mr. Augustine Goba talked about laboratory capabilities, including the new generation of Lassa immunodiagnostics (rapid tests), with highlight opportunities for collaboration and capacity building

4. Integrate clinical and basic research findings to identify the most critical gaps in our knowledge and understanding of LF and what steps could aid future studies.
This aim was achieved. It was resolved to correct mistakes in lab investigations and diagnostic analyses that happened during the Ebola outbreak, and to build capacity on laboratory and diagnostic services and strengthen surveillance systems. We also showcased opportunities for fieldwork in Kenema district.

5. Promote collaborations to move LF research, including vaccine and therapeutic development, forward at a faster pace. Develop creative solutions to unresolved challenges in research and implementation of LF policies and programs.
This aim was achieved. The aims and objectives of the training: Ensure quality assurances and increased knowledge on laboratory and diagnosis in Sierra Leone by improving the survival of the population on the Ebola outbreak and other Hemorrhagic fevers.

6. Provide funds to graduate students, postdoctoral trainees, junior faculty members, and members of under-served and under-represented stakeholder groups for the purpose of attending this conference.

This aim was achieved. Attendance came from the National level, WHO, CDC, MSF and partners. Approximately half of the attendees were students or junior scientists.

7. Create a setting that will contribute to the probability of success of the conference, and provide adequate support, equipment and other physical resources.
This aim was achieved. The meeting was held at the Barmoi Hotel with 35 people in attendance. The composition of the audience was a diverse mix of Americans, Europeans, Asians and Africans.

List of publications.
Not applicable

The final progress report also should address the following when applicable:
1. Report on the final enrollment data for study subjects based on sex/gender, race, and ethnicity (use the PHS Inclusion Enrollment Report).
Not applicable

2. If appropriate, indicate whether children were involved in the study or how the study was relevant for conditions affecting children (see Public Policy Requirements and Objectives—Inclusion of Children as Subjects in Clinical Research).
Not applicable

3. Describe any data, research materials (such as cell lines, DNA probes, animal models), protocols, software, or other information resulting from the research that is available to be shared with other investigators and how it may be accessed. If the initial research plan addressed, or the terms of award require, a formal plan for sharing final research data, model organisms, Genome Wide Association Studies data, or other such project-specific data, provide a final statement on the implementation of that plan.
Not applicable

4. Publications that were authored or co-authored by the PD/PI and arose from the award must include the NIH Manuscript Submission reference number (e.g., NIHMS97531) or the PubMed Central (PMC) reference number (e.g., PMCID234567) for each article. If the PMCID is not yet available because the Journal submits articles directly to PMC on behalf of their authors, indicate "PMC Journal - In Process."
Not applicable

5. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the final progress report.
Not applicable

Cross section of speakers including Prof. Garry on the far right

PI: **Garry, Robert F**	Title: Opening a New Lassa Fever Ward in Kenema Sierra Leone	
Received: 04/12/2012	FOA: PA10-071	Council: 10/2012
Competition ID: ADOBE-FORMS-B1	FOA Title: NIH SUPPORT FOR CONFERENCES AND SCIENTIFIC MEETINGS (PARENT R13/U13)	
1 R13 AI104216-01	Dual: TR	Accession Number: 3484035
IPF: 8424601	Organization: TULANE UNIVERSITY OF LOUISIANA	
Former Number:	Department: Microbiology and Immunology	
IRG/SRG: ZAI1 KP-M (S1)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 100,000	Animals: N Humans: N Clinical Trial: N Current HS Code: Evaluative Info HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Robert Garry	Tulane University	PD/PI

OMB Number: 4040-0001
Expiration Date: 06/30/2011

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. * TYPE OF SUBMISSION
☐ Pre-application ☒ Application ☐ Changed/Corrected Application

2. DATE SUBMITTED: 04/12/2012
Applicant Identifier:

4. a. Federal Identifier:
b. Agency Routing Identifier:

5. APPLICANT INFORMATION
*** Organizational DUNS:** 053785812

* Legal Name: Tulane University
Department: Sponsored Projects Admin
Division:
* Street1: 1430 Tulane Avenue, EP-15
Street2:
* City: New Orleans
County / Parish: Orleans
* State: LA: Louisiana
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 70012-2632

Person to be contacted on matters involving this application
Prefix: Ms.
* First Name: Kathleen
Middle Name: M
* Last Name: Kozar
Suffix:
* Phone Number: 5049885613
Fax Number: 5049881748
Email: elecnotf@tulane.edu

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN)*: 720423889

7. * TYPE OF APPLICANT: O: Private Institution of Higher Education
Other (Specify):
Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:
☒ New ☐ Resubmission
☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).
☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration
☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY: National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:
TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
Opening a New Lassa Fever Ward in Kenema Sierra Leone

12. PROPOSED PROJECT:
* Start Date: 12/01/2012
* Ending Date: 11/30/2013

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT**: LA-002

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION
Prefix: Prof.
* First Name: Robert
Middle Name: F
* Last Name: Garry
Suffix:
Position/Title: Professor/Assistant Dean
* Organization Name: Tulane University
Department: Microbiology and Immunology
Division: Medicine
* Street1: 1430 Tulane Avenue
Street2:
* City: New Orleans
County / Parish: Orleans
* State: LA: Louisiana
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 70112-2632
* Phone Number: 5049882027
Fax Number: 5049881994
* Email: rfgarry@tulane.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	100,000.00
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	100,000.00
d. Estimated Program Income	0.00

16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalities. (U.S. Code, Title 18, Section 1001)

☒ *** I agree**

**** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.***

18. SFLLL or other Explanatory Documentation

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: Ms. * First Name: Kathleen Middle Name: M

* Last Name: Kozar Suffix:

* Position/Title: Director

* Organization: Tulane University

Department: Sponsored Projects Admin. Division:

* Street1: 1430 Tulane Avenue, EP-15

Street2:

* City: New Orleans County / Parish: Orleans

* State: LA: Louisiana Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 70112-2632

* Phone Number: 5049885613 Fax Number: 5049881748

* Email: kkozar@tulane.edu

*** Signature of Authorized Representative**: Kathleen Kozar

*** Date Signed**: 04/12/2012

20. Pre-application Add Attachment | Delete Attachment | View Attachment

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 08/31/2011

Project/Performance Site Location(s)

Project/Performance Site Primary Location ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Tulane University

DUNS Number: 0537858120000

* Street1: 1430 Tulane Avenue

Street2:

* City: New Orleans County: Orleans

* State: LA: Louisiana

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 70112-2699 * Project/ Performance Site Congressional District: LA-002

Project/Performance Site Location 1 ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:

DUNS Number:

* Street1:

Street2:

* City: County:

* State:

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: * Project/ Performance Site Congressional District:

Additional Location(s) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☐ Yes ☒ No

1.a If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☐ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? ☐ Yes ☒ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. * Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. * Does this project involve activities outside of the United States or partnerships with international collaborators? ☒ Yes ☐ No

6.a. If yes, identify countries: Sierra Leone

6.b. Optional Explanation:

7. * Project Summary/Abstract 1236-Lassa Ward Conference Abstract.p Add Attachment Delete Attachment View Attachment

8. * Project Narrative 1237-Lassa Ward Conference Narrative. Add Attachment Delete Attachment View Attachment

9. Bibliography & References Cited 1238-Lassa Ward Conference Bibliograp Add Attachment Delete Attachment View Attachment

10. Facilities & Other Resources 1239-Resources KGH red.pdf Add Attachment Delete Attachment View Attachment

11. Equipment Add Attachment Delete Attachment View Attachment

12. Other Attachments Add Attachments Delete Attachments View Attachments ☐

Lassa fever (LF) is an acute and often fatal hemorrhagic disease that is endemic in the West African countries of Sierra Leone, Guinea, Liberia and Nigeria. Kenema Government Hospital (KGH) in Sierra Leone, West Africa is a 350-bed facility situated in the heart of the region with the highest incidence in the world of LF. Because of the importance of LF as a bioterrorism and public health threat, KGH has developed an advanced clinical and laboratory research capacity. KGH currently maintains a 25-bed Lassa Ward and full-time staff completely dedicated to the care of patients with LF. Recently, the KGH research team has broken ground on a new 48-bed Lassa Ward that will replace this historic, but timeworn facility. This is a request for support of a one-time NIAID conference in association with opening of the new Lassa Ward in Sierra Leone. The Specific Aims of our Conference Plan are to 1. Commemorate the opening of the new Lassa Ward, reflect on the historical, societal and scientific impact of LF, and increase public awareness of the continued impact of LF in Sierra Leone and the West African subregion; 2. Assemble several generations of experts on LF who may otherwise not have an opportunity to meet to share and debate their latest findings and to define goals for the future; 3. Present training exercises and workshops to showcase new capabilities in West Africa for LF clinical and laboratory research; 4. Integrate clinical and basic research findings to identify the most critical gaps in our knowledge and understanding of LF and what steps could aid future studies; 5. Promote collaborations to move LF research, including vaccine and therapeutic development, forward at a faster pace. Develop creative solutions to unresolved challenges in research and implementation of Lassa fever policies and programs; 6. Provide funds to graduate students, postdoctoral trainees, junior faculty members, and members of under-served and under-represented stakeholder groups for the purpose of attending this conference, and 7. Create a setting that will contribute to the probability of success of the conference, and provide adequate support, equipment and other physical resources. We will discuss the many recent breakthroughs in our understanding of the virology, immunology, epidemiology and natural history of Lassa fever. In this open meeting we hope to bring together experts on LF from several generations of scientists and to cover a broad range of subject matter. We will invite anyone interested to attend, while maintaining uniform standards for selecting the final list of participants. We anticipate that scientists from across West Africa as well as from the United States, South America, Europe, Asia and elsewhere will attend this timely conference.

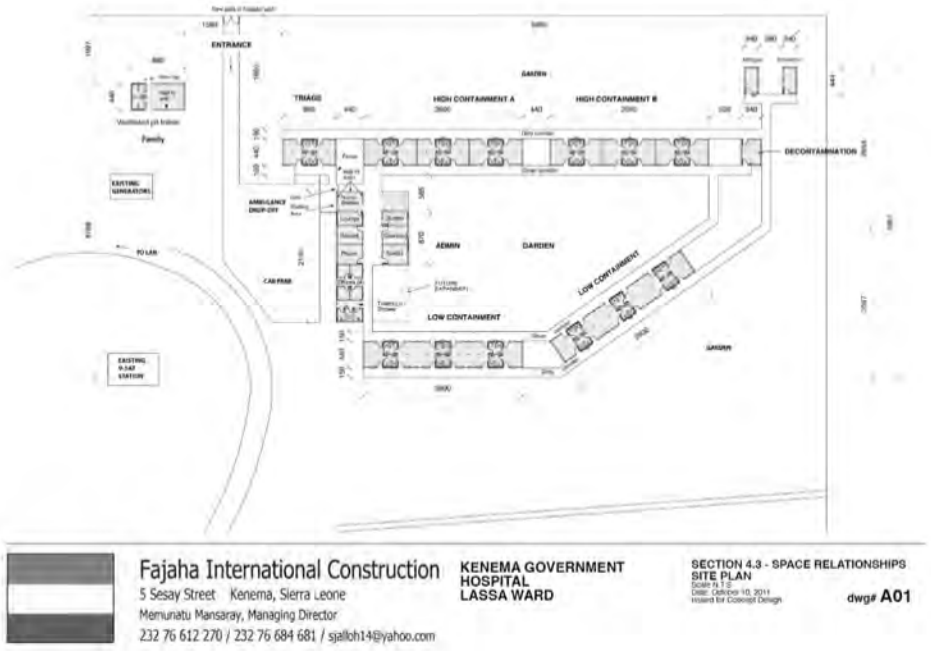
This is a request for support of a one-time NIAID conference in association with opening of the new Lassa Ward in Sierra Leone. We will discuss the many recent breakthroughs in our understanding of the virology, immunology, epidemiology and natural history of Lassa fever. In this open meeting we hope to bring together experts on LF from several generations of scientists and to cover a broad range of subject matter.

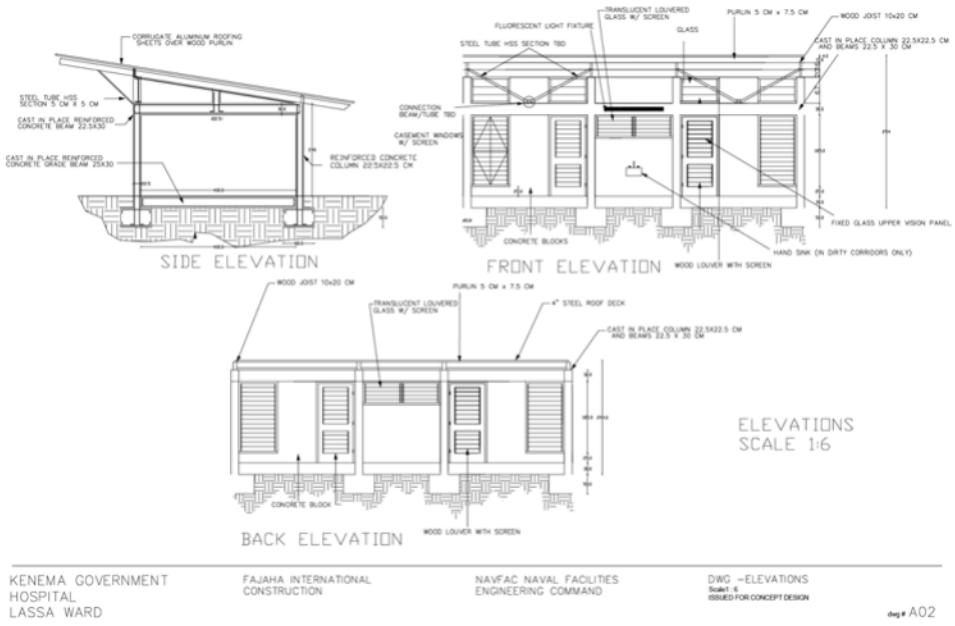
Kenema Government Hospital (KGH), a the 350-bed referral hospital for Kenema District in eastern Sierra Leone, maintains a year-round 25-bed ward for the care of patients with Lassa Fever. The ward is staffed with a full-time team of doctors, nurses, and technicians. Recently, construction has been initiated on a new 48-bed Lassa Ward.

The original Lassa Ward

Location of New Lassa Ward (Google Earth)

Design of New Lassa Ward





Typical Construction at KGH

A laboratory has been established in Kenema for diagnosis for Lassa fever and other viral diseases. Samples are manipulated in class II biosafety cabinets by personnel wearing full personal protective materials (gowns, gloves, and mask). The KGH Lassa Laboratory is located on the grounds of the hospital, but in a stand-alone building constructed in 2005. The laboratory is comprised of an approximately 5,500 square foot building divided into a general clinical laboratory for routine diagnostics and a 700 square-foot specialized suite for manipulation of samples from suspected cases of Lassa fever.. ELISA (antigen and IgM and IgG antibody) and real-time PCR for Lassa fever are performed here. Access to the building and the BSL-3 suite is controlled and recorded. Negative airflow is maintained. The laboratory possesses equipment and trained personnel for diagnostics using real-time PCR (with separate PCR suites, ELISA, and immunofluorescent antibody tests. For safety reasons, no cell culture is performed. The building is equipped with redundant power sources, including town power in Kenema (which is extremely sporadic), a new solar power system (panels, batteries, controllers and inverters, and 100, 16 and 6 kilovolt generators. Established biosafety and biosecurity guidelines are maintained, with oversight by the Sierra Leone Ministry of Health and Sanitation, WHO and Tulane. In March 2008 the KGH Lassa Laboratory underwent site review by NIH Program Officers and was found acceptable.

LASSA LABORATORY LAB TEAM From Left to Right:
Mambu Momoh. Mohammed Fullah, Augustine Goba,

The Kenema Government Hospital Lassa Laboratory. The solar panels (additional panels that will cover the sun-facing part of the roof have now been installed) provide essential power for refrigerators, freezers and other essential instruments. The Kenema Government Hospital Lassa Ward is behind the buildings up the hill to the left, not visible in this view.

Sample Processing in the Kenema Government Hospital Lassa Laboratory. Samples are manipulated in class II biosafety cabinets by personnel wearing full personnel protective materials.

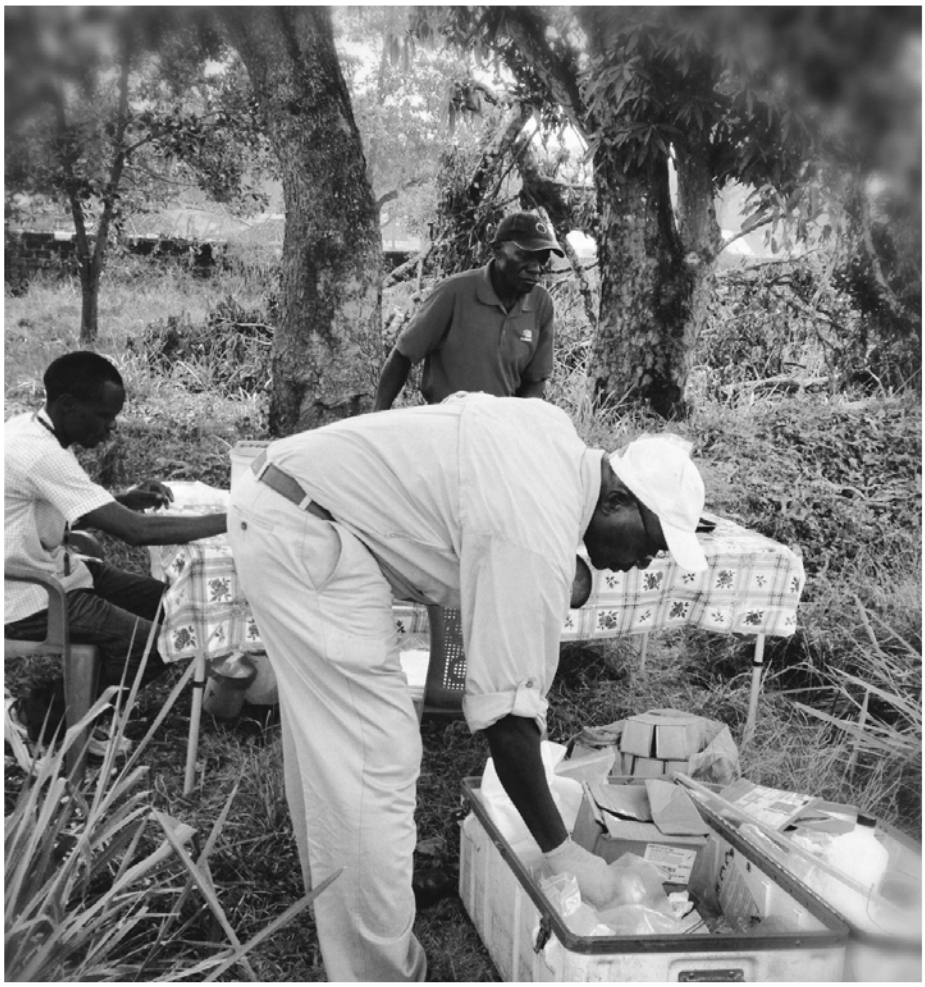
The structure shown, which is adjacent to the KGH laboratory, is constructed from prefabricated office containers donated by the United Nations, some of which have been subsequently refitted to serve as PCR clean rooms. A 2006 Toyota Land Cruiser (shown, seating 90) and two Toyota Prados (seating 5) are available for use at the Conference. An 2012 2006 Toyota Land Cruiser (seating 9) is in transit to the sight at this writing and will also be available for use at the conference.

Equipment in the Lassa Laboratory. 1. ELISA plate reader, 2. Lightcycler. 3. Flow cytometer, 4. Cell culture incubator, 5. class 2 hoods, 6. SatCom high speed Internet.

Dr. Donald S. Grant, Director Lassa Fever Program at KGH, MOHS

Lassa Fever Program Coordinator at KGH: Simbirie Jalloh

LASSA FEVER ECOLOGY TEAM



From Left to Right: Willie Roberts, Kandeh Karbo (front), James Koninga (back)

Willie Roberts

Outreach Team

The outreach team is comprised of Lansana Kanneh and Richard Fonnie. Both have more than a decade of experience in community health and promotion. Previously they worked for the NGO Merlin on its outreach team, conducting Lassa fever work. Each has specific roles and responsibilities that they have honed throughout their long history of outreach activities. Richard has a talent in engaging leaders and important people of the community. Lansana is known as the Mende (local language) Encyclopedia because of his linguistic skills and cultural knowledge and gives all of the large group talks. He is a gifted speaker and communicates with cultural precision. Additional team members from the Kenema Government Hospital Lassa fever team may assist when a third member is needed for activities.

Richard Fonnie

Community Selection

Locations for community collections are selected based on several variables:

1. If follow-up for a case (which occurs at 2 weeks, 3 months and 6 months after release from the hospital) is needed and the community meets the inclusion criteria.
2. If contract tracing of a current case is needed and the community meets the inclusion criteria.
3. If the community was visited in the past, was open to a return visit for additional studies, and meets the inclusion criteria.

Operations

Once villages are selected, a driver takes the outreach team out to the village. They often stay from one to several nights. Villages are often remote and roads and transportation offers a great challenge. The Chief and elders of the community are first addressed upon arrival to the village. Often additional community members also gather and listen to the outreach team explain their purpose and ask for approval to conduct the studies. A community talk is given after nightfall. Its focus is on Lassa fever sensitization and an explanation of any studies that will take place. The talk runs for somewhere between 1 and 2 hours and includes the CDC video on Lassa fever.

The following is an example dialog roughly translated from Mende to English.

Example Talk (translated from Mende)

We are from the Kenema Government Hospital. We are in the community because of Lassa fever. We are here to talk about a couple of things. The first is what is the disease and how can you avoid getting it. The second is to tell you about a couple of studies we are currently doing.

Lassa fever is very severe but this isn't new to everyone here. Since we are here for a follow-up of a case, many of you may have already heard about Lassa. Our first priority

for being in the community is because someone in the community has had Lassa and was at the ward in Kenema. We want to check the health of that person and see their living conditions.

Lansana Kanneh

We are here to not only tell you about Lassa, signs and symptoms and prevention; but there are lots of questions about Lassa. People ask about vaccines but we can't answer these questions without your help and cooperation. We are here to learn from you and do a study. We would like for you to give samples for this study. There is no money and no direct benefit, but later good things may come from it.

History of Lassa fever: When it was found in Sierra Leone and a bit about its origins in Nigeria. When Lassa was discovered in Sierra Leone the Ministry of Health and other organizations came together to try to figure out where the disease came from and who was getting it. (*This is to show that many people are invested in the research and that there help is needed and appreciated*). After that research, it was discovered that Lassa is found in the rats. That is spread through urine and feces through food and in houses. Rat to Man is the primary source. (*The transmission route is described in more detail*)

(*Creating a scenario to understand transmission*) If you eat the rat you can get Lassa. People think that if they cook it well they are safe. But the person who prepares it is not safe. They are touching the blood and they could get Lassa.

Human to human transmission is the second route of transmission. Once the virus gets into the human then the human fluids also have the Lassa virus and is dangerous. *(The secondary transmission route is described in more detail)*

Signs and symptoms of the disease:
Typically it appears like any other fever case like Malaria and typhoid. There are stages in Lassa fever. The last would be convulsion, coma and death. It doesn't all just come at one time. It may take 6 to 21 days to occur and build up. If this person goes through all of these symptoms and dies in the community, they will leave you all with the disease. When you care for the body after death you can get the virus. Some people say that it is witchcraft and other things but no, it is the virus from the body.

Prevention and control Methods:
We are seeing more women and children with Lassa fever. The women prepare the food

and when they get items from the market, it is sold from places that are not rodent free. They often eat the food raw and give it to the children. This is putting women and children at risk. Pregnancy and Lassa is very, very dangerous. The survival rate in cases like this very small. It can also cause permanent deafness in some cases.

Before 1972 there was Lassa fever even if we didn't call it Lassa fever. There was an outbreak from 1952 to 1954 in Yengema. This fever was known as Yengema fever but it is most likely that is was Lassa fever. Even though you have been eating rats for a long times, and we didn't call it Lassa fever doesn't mean it wasn't Lassa fever. *(Often the community brings up that rats have been eaten for a long time and Lassa appears to be new. He is addressing this common question.)*

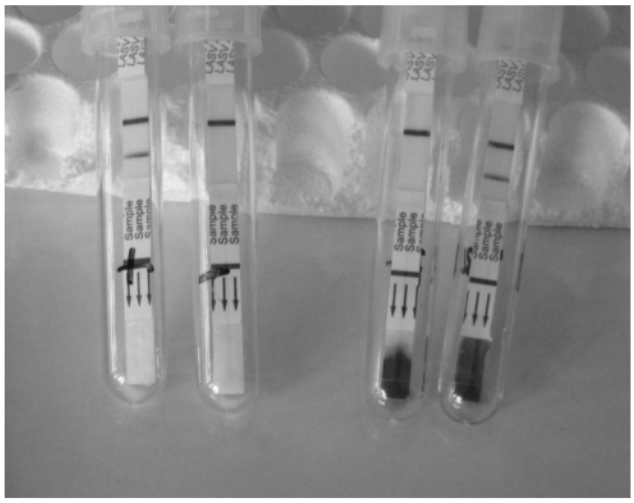
Just like HIV is very severe so is Lassa. With HIV it is hard to get drugs, with Lassa drugs are available and free. If rats are responsible then everyone should try to keep them out of their homes. You should use the traditional trap, keep cats and use good hygiene. If it looks like rodents have eaten something throw it away. Cover your food and water. It is very important to refer all of your fever cases to community health center. Then they can be screened and treated if they have Lassa for free. This will also act as a control so one case cannot cause another. We know that you are poor and that your houses are mud but everyone should try to control rodents the best that they can.

Panguma and Segbwema missionary hospitals trained as satellite sites for LF Rapid Test clinical trials. All have high referral rates to Kenema Lassa Ward.

Panguma Mission Hospital

Signs in Panguma Mission Hospital

Lassa Rapid Test Training in Panguma



Segbwema

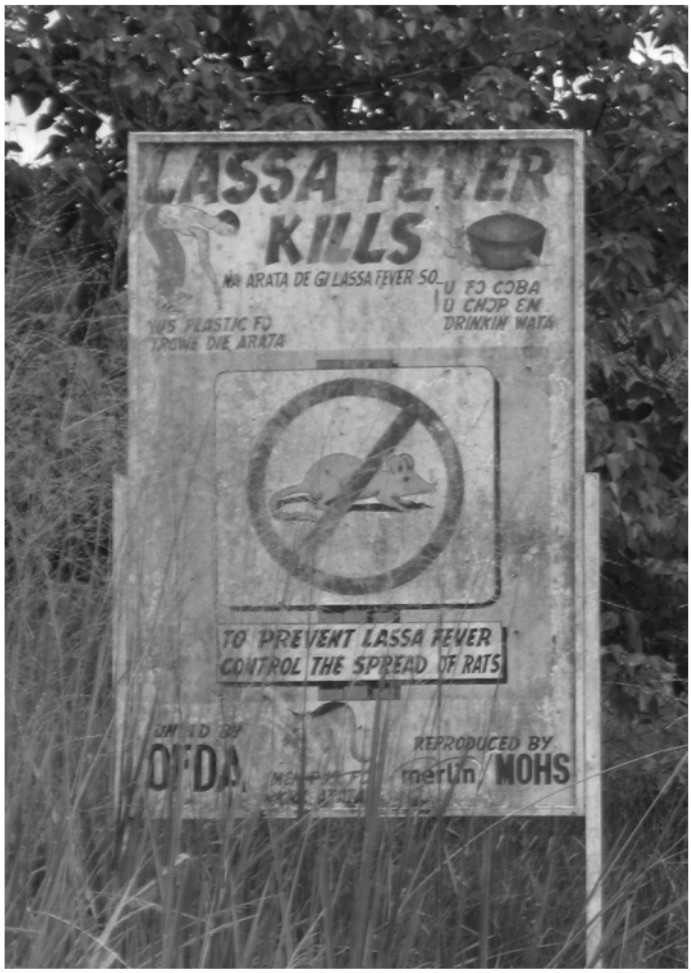
Nixon Mission Hospital at Segbwema

New Conference Hall under construction at Kenema City Council complex. Estimated time of completion is May 2012.

Outside construction of the new hall at Kenema City Council

Kenema City Council Building, which is adjacent to new Hall.

Kamboi Hall banquet facility in Kenema Sierra Leone


LASSA FEVER
KILLS
NA ARATA DE GI LASSA FEVER SO
U FO COBA
U CHOP EM
DRINKIN WATA
TO PREVENT LASSA FEVER
CONTROL THE SPREAD OF RATS
REPRODUCED BY
MOHS

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Prof. * First Name: Robert Middle Name: F
* Last Name: Garry Suffix:
Position/Title: Professor/Assistant Dean Department: Microbiology and Immunology
Organization Name: Tulane University Division: Medicine
* Street1: 1430 Tulane Avenue
Street2:
* City: New Orleans County/ Parish: Orleans
* State: LA: Louisiana Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 70112-2632
* Phone Number: 5049882027 Fax Number: 5049881994
* E-Mail: rfgarry@tulane.edu

Credential, e.g., agency login: eRA Commons User Name

*** Project Role:** PD/PI **Other Project Role Category:**

Degree Type: PHD
Degree Year: 1978

***Attach Biographical Sketch** 1234-Garry biosketch Lassa Wa [Add Attachment] [Delete Attachment] [View Attachment]
Attach Current & Pending Support 1235-Garry Other Support Lass [Add Attachment] [Delete Attachment] [View Attachment]

PROFILE - Senior/Key Person 1

Prefix: * First Name: Middle Name:
* Last Name: Suffix:
Position/Title: Department:
Organization Name: Division:
* Street1:
Street2:
* City: County/ Parish:
* State: Province:
* Country: USA: UNITED STATES * Zip / Postal Code:
* Phone Number: Fax Number:
* E-Mail:

Credential, e.g., agency login:

*** Project Role:** **Other Project Role Category:**

Degree Type:
Degree Year:

***Attach Biographical Sketch** [Add Attachment] [Delete Attachment] [View Attachment]
Attach Current & Pending Support [Add Attachment] [Delete Attachment] [View Attachment]

BIOGRAPHICAL SKETCH

NAME Robert F. Garry	POSITION TITLE Professor, Microbiology and Immunology
eRA COMMONS USER NAME (credential, e.g., agency login)	Assistant Dean for Graduate Studies in Biomedical Sciences

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Indiana State University, Terre Haute, Indiana	B.S.	1974	Life Sciences
University of Texas, Austin, Texas	Ph.D.	1978	Microbiology
University of Texas, Austin, Texas	Postdoctoral		Virology

A. Personal Statement

Research in the Garry Laboratory focuses on a number of aspects of viral pathogenesis. He is currently managing a consortium of scientists who are developing modern immunodiagnostics for Lassa virus and several other biodefense pathogens. Dr. Garry also has a long-standing interest in viral diagnostics dating back to his work with the industry scientists who developed the first generation of ELISA and western blot assays to detect serum antibodies to HIV-1. He also continues to main a broad interest in the mechanisms of enveloped virus entry. He was involved in collaborative studies that lead to the determination that entry proteins of enveloped viruses form at least three distinct structural classes. This work was the foundation for the development of novel antiviral peptide drugs, of which the AIDS drug Fuzeon (enfuvirtide) was the first-in-class. Dr. Garry has published over 180 papers, book chapters or reviews in virology and related areas. He also serves in the Tulane University administration as Assistant Dean for Graduate Studies in Biomedical Sciences. He established (with Dr. David Sander) All the Virology on the World Wide Web (www.virology.net), one of the first Internet index sites for virology, which remains popular today. He is the founding Editor-in-Chief of *Virology Journal* (Biomed Central; www.virologyj.com), and has served on many NIH review panels. He has organized or served on the organizing committees of numerous scientific conferences.

B. Positions and Honors.

1983 - date Assistant Professor (1983-87); Associate Professor (1987–93); Professor (1993-date) of Microbiology and Immunology at Tulane University School of Medicine at New Orleans, Louisiana 70112.
1995 – date Established (with Dr. David Sander) All the Virology on the World Wide Web.
2006 – date Assistant Dean for Graduate Studies in Biomedical Sciences
2004 – date Editor-in-Chief: *Virology Journal* (BioMed Central)
1988 - date Member of 6 *Ad hoc* NIH AIDS Study Sections (ARR-3, 1988-90, before charter); Member of NIH AIDS Molecular Biology Study Section (ARR-C, 1990-1996); Member or Chair of over 40 NIAID, NHLBI, MBRS, NCI or HHS Special Study Sections (1996-date). Chair, VATID Biodefense Study Section (2002); Chair, SBIR-STTR Biodefense Study Section (2002-2006), Chair, special AIDS vaccine Study Sections (2009, 2010); Member, SBIR-STTR Non-HIV Anti-infectives Study Section (2006-2010).

C. Selected Peer-reviewed Publications (Selected from >180 peer-reviewed publications)

Most relevant to the current application

Branco, L., Matschiner, A., Fair, J.N., Goba, A., Ferro, P., Cashman, K., Sampey, D., Schoepp, R., Tesh, R., Bausch, D.B., Garry, RF, and Guttieri, M.C. (2008). Bacterial-based systems for expression and purification of recombinant Lassa virus proteins of immunological relevance. *Virol J.* **5,** 73. PMCID: PMC2435526

Illick M.M., Branco, L.M., Fair, J.N., Illick, K.A, Matschiner, A., Schoepp, R., Garry, R.F., Guttieri, M.C. (2008). Uncoupling GP1 and GP2 expression in the Lassa virus glycoprotein complex: implications for GP1 ectodomain shedding. *Virol J* **5,** 161. PMCID: PMC2645378

Branco,, L.M., and Garry, RF. (2009). Characterization of the Lassa virus GP1 ectodomain shedding: implications for improved diagnostic platforms. *Virol J* **6,** 147. PMCID: PMC2759938

Branco LM, Grove JN, Geske FJ, Boisen ML, Muncy IJ, Magliato SA, Henderson LA, Schoepp RJ, Cashman KA, Hensley LE, Garry RF. (2010) Lassa virus-like particles displaying all major immunological determinants as a vaccine candidate for Lassa hemorrhagic fever. *Virol J.* **7,** 279. PMCID: PMC2984592

Branco LM, Grove JN, Moses LM, Goba A, Fullah M, Momoh M, Schoepp RJ, Bausch DG, Garry RF. (2010) Shedding of soluble glycoprotein 1 detected during acute Lassa virus infection in human subjects. *Virol J.* **7,** 306. PMCID: PMC2993672

Additional recent publications of importance to the field (in chronological order)

Rasheed, S., A. A. Gottlieb, and R.F. Garry (1986). Cell killing by UV-inactivated human immunodeficiency virus. *Virology* **153,** 395-400.

Gallaher, W.R., Ball, J.M., Garry, R.F., Griffin, M.C., and Montelaro, R.C. (1989). A general model for the TM proteins of HIV and other retroviruses. *AIDS Res. Hum. Retro.* **5,** 431-440.

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Garry, R.F., and Witte, M. (1990). Early case of AIDS in the United States. *Nature* **347,** 509.

Qureshi, M.N, Coy, D.H., Garry, R.F., and Henderson, L.A. (1990). Characterization of a putative cellular receptor for the HIV-1 TM glycoprotein using synthetic peptides. *AIDS* **4,** 553-558.

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Miller, M.A., Garry, R.F., Jaynes, J.M., and R.C. Montelaro. (1991). Possible structural correlation between lentivirus transmembrane proteins and natural cytolytic peptides. *AIDS Res. Hum. Retro.* **7,** 511-519.

Garry, R.F., and Fermin, C.D. (1993). Viral burden in AIDS. *Nature* **365,** 301-302, 1993.

Voss, T.G., Fermin, C.D., Levy, J.A., Vigh, S., Choi, B., and Garry, R.F. (1996). Alteration of intracellular Na+ and K+ concentrations correlates with induction of cytopathic effects by HIV. *J. Virol*., **70,** 5447-5454.

Choi, B., Gatti, P.J., Haislip, A.M., and Garry, R.F. (1998). Role of potassium in HIV production and cytopathic effects. *Virology* **247,** 189-199.

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Plymale, D.R., Comardelle, A., Fermin, C.D., Ng Tang, D., Lewis, D.E, and Garry, R.F. (1999). HIV-1 kills cells using both apoptotic and necrotic pathways. *AIDS* **13,** 1827-1839.

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Garry, R. F. and Dash, S. (2003). Proteomics computational analyses suggest that hepatitis C virus E1 and pestivirus E2 envelope glycoproteins are truncated class II fusion proteins. *Virology* **307,** 255-265.

Sainz, B. Jr., Mossel, E.C., Peters, C.J. and Garry, R.F. (2004). Interferon-b and interferon-g synergistically inhibit replication of severe acute respiratory syndrome-associated coronavirus. *Virol.* **329,** 11-17.

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Jaspan, H.B., Robinson, J.E., Amedee, A.M., Van Dyke, R.B., and Garry, R.F. (2004). Neutralizing antibody to HIV-1 and SIV in amniotic fluid. *J. Clin. Virol.* **31,** 190-197.

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Tenenbaum, S.A., Morris, C.A., Alexander, S.S., McFerrin, H.E., Garry R.F.,and Leissinger C.A. (2005). Evidence of HIV exposure and transient seroreactivity in archived HIV-negative severe hemophiliac sera. *Virol J*. **2,** 65.

Cabrera-Batista, B., Skewes-Ramm, R., Fermin, C.D., Garry, R.F. (2005). Dengue in the Dominican Republic: epidemiology for 2004. *Microsc Res Tech*. **68,** 250-4.

Hrobowski Y.M., Garry R.F., and Michael S.F. (2005). Peptide inhibitors of dengue virus and West Nile virus infectivity. *Virol J* **2,** 49.

Mossel, E.C., Sainz, B. Jr, Garry, R.F., and Peters, C.J. (2006). Synergistic inhibition of SARS-coronavirus replication by type I and type II IFN. Adv Exp Med Biol. **581,** 503-6.

Colmegna, I., Koehler, J.W., Garry, R.F., and Espinoza, L.R. (2006). Musculoskeletal and autoimmune manifestations of HIV, syphilis and tuberculosis. *Curr Opin. Rheumatol.* **18,** 88-95.

Costin, J.M., Rausch, J.M., Garry, R.F., and Wimley, W.C. (2007). Viroporin potential of the lentivirus lytic peptide (LLP) domains of the HIV-1 gp41 protein. *Virol J.* **4,** 123.

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Koehler, J.W., Bolton, M., Rollins, A., Snook, K., deHaro, E., Henson, E., Rogers, L., Martin, L.N., Krogstad, D.J., James, M.A., Rice, J., Davison, B., Veazey, R.S., Prabhu, R., Amedee, A.M., Garry, R.F., Cogswell, F.B. (2009). Altered immune responses in rhesus macaques co-infected with SIV and Plasmodium cynomolgi: an animal model for coincident AIDS and relapsing malaria. *PLoS One* **4,** e7139. PMCID: PMC2744481

Safronetz D., Lopez J.E., Sogoba N., Traore' S.F., Raffel S.J., Fischer E.R., Ebihara H., Branco L., Garry R.F., Schwan T.G., Feldmann H. (2010). Detection of Lassa virus, Mali. *Emerg Infect Dis* **16,** 1123-6.

Hazari S, Chandra PK, Poat B, Datta S, Garry RF, Foster TP, Kousoulas G, Wakita T, Dash S. (2010). Impaired antiviral activity of interferon alpha against hepatitis C virus 2a in Huh-7 cells with a defective Jak-Stat pathway. *Virol J* **7,** 36. PMCID: PMC2831880

Chandra P.K., Hazari S., Poat B., Gunduz F., Prabhu R., Liu G., Burioni R., Clementi M., Garry R.F., Dash S. (2010). Intracytoplasmic stable expression of IgG1 antibody targeting NS3 helicase inhibits replication of a highly efficient hepatitis C Virus 2a clone. *Virol J* **7,** 118. PMCID: PMC2903558

Sabahi A. Marsh K.A., Dahari H., Corcoran P., LaMora J.M., Yu X., Garry R.F., Uprichard S. L. (2010). The rate of hepatitis C virus infection initiation in vitro is directly related to particle density. *Virology*, **407,** 110-9. PMCID: PMC2946418

Bolton M.J., Garry R.F. (2011). Sequence similarity between the erythrocyte binding domain 1 of the Plasmodium vivax Duffy binding protein and the V3 loop of HIV-1 strain MN reveals binding residues for the Duffy Antigen Receptor for Chemokines. *Virol J.* **8,** 45. PMCID: PMC3040140

Melnik L.I. Garry R.F., Morris C.A. (2011). Peptide inhibition of human cytomegalovirus infection. *Virol J.* **8,** 76. PMCID: PMC3050824

Gaston A, Garry RF. (2012) Topical vitamin A treatment of recalcitrant common warts. Virol J. **9,** 21. PMCID: PMC3274422

D. Research Support

ACTIVE

1U01AI082119-01 Garry (PI) 06/01/09 - 05/31/14
NIAID
Preclinical development of recombinant antigen diagnostics for Lassa fever
The goal is to complete the preclinical development phase for recombinant IgM-, IgG- and antigen-capture assays and point-of-care lateral flow assays for diagnosis of infection by Lassa virus. This is the current application.
Role: PI

RC-0013-07 Garry (PI) 9/1/07 – 7/31/11
Louisiana Board of Regents
Design, Delivery and Development of Therapeutic Peptides
This Program Project style grant is a consortium of seven projects and three cores to develop peptide based drugs for treatment of infectious diseases, immunological diseases, cardiovascular disease and cancers.
Role: Dr. Garry is the overall PI, and director of one Project on biothreat viruses and the Administrative Core.

R44 AI082778-01 (PI: Wilson) 04/01/09 - 3/30/12
NIAID
Peptide inhibitors of influenza entry-FAST TRACK
Synthetic peptide inhibitors of influenza virus entry will be developed and tested in vitro and in vivo.
Role: Director of subcontract

HHSN272200900049C (PI-Robinson) 09/30/09 – 09/29/14
NIAID-DAIT
Roles of protective or pathogenic B cell epitopes in human Lassa fever
The goals are to discover novel B cell epitopes of Lassa virus protein antigens and to elucidate mechanisms of antibody-mediated protection or pathogenesis in a well-characterized cohort of persons exposed to diverse strains of LASV at different stages and with different severities of Lassa fever.
Role: Program Manager responsible for overall scientific and logistical management of the project.

R44 AI082805-01 (PI: Geske) 04/01/2010 - 03/31/12
NIAID
Recombinant antigen diagnostics for filoviruses
The goal is to develop modern IgM-, IgG- and antigen-capture assays and point-of-care lateral flow assays for diagnosis of infection by filoviruses.
Role: Director of subcontract

HHSN272201000022C (Co-PIs: Sabeti and Garry) 10/01/10 – 09/30/15
NIAID
Host Genetic Factors in Resistance to Lassa Hemorrhagic Fever
The goals of this Project are to replicate strong signals of natural selection found in Yorubans at genes critical for infection with Lassa virus (LASV) in four additional West African populations and to localize and characterize the key functional mutations.
Role: Director of subcontract.

Competed Research Support (Last 3 years)

1UC1AI067188-01 Garry (PI) 7/1/05 – 6/30/08
NIAID
Recombinant antigen multiagent diagnostic assays for Lassa and other arenaviruses
Role: PI

The goals are to develop modern IgM-, IgG- and antigen-capture assays for diagnosis of infection by Old and New World arenaviruses. 1U01AI082119-01 continues the preclinical development of these assays

PENDING
None

OVERLAP
Dr. Garry will not accept total funding over [EFFORT] calendar months.

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 0537858120000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Tulane University

Delete Entry *** Start Date:** 12/01/2012 *** End Date:** 11/30/2013 **Budget Period** 1

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Prof.	Robert	F	Garry		PD/PI	0.00	0.00			0.00	0.00	0.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 0.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
	Total Number Other Personnel					**Total Other Personnel**	

Total Salary, Wages and Fringe Benefits (A+B) 0.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 0537858120000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Tulane University

Delete Entry * Start Date: 12/01/2012 * End Date: 11/30/2013 Budget Period 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	Total funds requested for all equipment listed in the attached file	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	86,500.00
4. Subsistence	
5. Other	

65 Number of Participants/Trainees Total Participant/Trainee Support Costs 86,500.00

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 1

Next Period

* ORGANIZATIONAL DUNS: 0537858120000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Tulane University

Delete Entry * Start Date: 12/01/2012 * End Date: 11/30/2013 Budget Period 1

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Logistical Support	13,500.00
9.	
10.	
Total Other Direct Costs	13,500.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	100,000.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	None			0.00
2.				
3.				
4.				
	Total Indirect Costs			0.00

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	100,000.00

J. Fee	Funds Requested ($)
	0.00

K. * Budget Justification 1243-Lassa Ward Conference Budget Just Add Attachment Delete Attachment View Attachment

(Only attach one file.)

Outline of Requested Expenses for Proposed Conference: Opening a New Lassa Fever Ward in Kenema Sierra Leone.			
Category	**Expense**	**Cost**	**Total**
Travel support	Invited speakers (pioneers)	6 @ $2500	$15,000
	Invited speakers (state-of-the-art)	12 @ $1500	$18,000
	African speakers (non Sierra Leonean)	6 @ $2000 (ave.)	$12,000
	Sierra Leone participants (nonKGH)	20 @ $200	$4,000
	Grad Student/Postdoc Travel Awards	15 @ $1500	$22,500
	Support Staff (US)	2 @ $2500	$5,000
	NIAID staff	4 @ $2500	$10,000
		Subtotal	**$86,500**
Logistical support	Buses/vehicle rental	$3500	$3500
	Conference Website	$1500	$1500
	Hall rentals	$3500	$3500
	Programs /printing	$500	$500
	Fuel	$1000	$1000
	PPE/other workshop supplies	$1500	$1500
	Childcare, other services	$500	$500
	Advertising	$1500	$1500
		Subtotal	**$13,500**
		Total	**$100,000**

Registration and Travel
We are requesting funds for partial support for 65 individuals to attend the conference in the following categories.

- *Invited speakers (pioneers).* Travel support along with a waiver of the registration fee is requested to allow 6 individuals from the first generation of scientists to work on Lassa fever in Sierra Leone to attend the conference. These individuals will provide an historical perspective on advances in the field, and offer unique insight into the challenges ahead. Health related issues might preclude some “pioneers” from attending. However, we expect at least six of these senior scientists to participate based on letters received or verbal commitments. Some individuals unable to participate in person will participate via a recorded video or live via “Skype”.

- *Invited speakers (state-of-the-art*). 12 invited speakers from the Americas, Europe or Asia will be provided support to partially cover travel expenses to the meeting. Please note that conference organizers from the VHFC will attend the meeting not be awarded travel funds from this request for conference support. These expenses will be covered in conjunction with

regular fieldwork conducted at the KGH site.

- *African speakers.* We will offer travel support along with a waiver of the registration fee to at least six African scientists from outside of Sierra Leone. Preference will be given to those individuals living or working within the LASV endemic range, principally from the countries of Guinea, Liberia and Nigeria. However, we will also consider travel awards for African scientists outside the Lassa range working on other arenaviruses or other viral hemorrhagic fevers on a case-by-case basis. We will vary the amount of the awards based on distance traveled. For example, an individual from neighboring Liberia will not require the same reimbursements as those from Nigeria.

- *Sierra Leone participants (non KGH).* Students from the two Universities in Sierra Leone, the University of Sierra Leone and Njala University will be offered a modest stipend ($200) and a waiver of the registration fee. This sum will cover travel expenses and lodging. Students may stay at the Pastoral Center in Kenema at a minimum cost (approximately $20 per night).

- *Graduate Student/Post-doctoral Fellow Travel Awards.* We anticipate making 15 awards to support partial travel expenses and a waiver of the registration fee for primarily to graduate students and post-doctoral fellows working on Lassa fever or other viral hemorrhagic fevers. Supported individuals will be selected on the basis of scholarship, need, and inclusion of women and under-represented minorities. While support will be mainly for graduate and post-doctoral students, we will also consider junior faculty or others, even senor scientists, that have been affected by the current research funding crisis.

- *Support Staff (US).* Simbirie Jalloh, KGH Program Coordinator for the VHFC will serve as the principal organizer on the ground in Kenema. Ms. Jalloh will be assisted in the task of organizing the meeting by several of the local KGH staff. Providing additional essential support both prior to and during to the meeting will be two administrators that have much experience at meeting organization. Emilie Broderick (Scripps Research Institute) and Ridhi Tariyal (Broad Institute/Harvard) will provide critical administrative support. Travel support and a waiver of the registration fee are requested for Ms. Broderick and Ms. Tariyal. The administrative personnel will assist the conference organizers by taking responsibility for the website, online registration and abstract collection, providing accurate financial records of the meeting's income and expenditures, coordinating the collection of all abstracts, preparing the program book, and communicating with registrants.

- NIAID staff. NIAID Conference Grants are cooperative agreements that have as an essential component the inclusion and participation of NIAID Staff. Travel support and a waiver of the registration fee are requested for four NIAID staff. Substantial NIH programmatic involvement with the awardees is anticipated during the further planning of the conference and during performance of the conference activities.

We estimate that travel expenses for attendees to the meeting will included air travel (typical fares $1300 to $1800 from the Americas, $1000 -$1200 from Europe). Participants that are to be supported will be required to travel on US flag carriers, if possible. Lungi Airport is the only commercial airport in Sierra Leone and it is located across a large estuary/bay about 30 miles from Freetown. We anticipate that some visitors will spend one or two nights in Freetown crossing from Lungi Airport to the Freetown suburb of Aberdeen, the location of several appropriate hotels. Transport from Lungi to Freetown is either by helicopter ($150, not recommended due to a spotty safety record), water taxi ($40) or ferry ($1.50). Conference attendees will be picked up either at Lungi Airport or Freetown (Aberdeen). An additional expense is hotels in Freetown and Kenema (5 nights at $130/night = $650).

Logistical Support

- Buses/vehicle rental. Visitors will be transported directly from Lungi Airport to Kenema via Port Loko via bus (an informative 6 hour drive passing though many typical villages and town). Alternatively, for those conferees spending a night in Freetown we will offer a bus from Freetown (Aberdeen hotels) to Kenema (approximately 4.5 hours).

- *Conference website.* Funds are requested to supplement the actual costs of providing a secure web site for registration and submitting abstracts. The viral hemorrhagic fever consortium website (vhfc.org) will host the conference registration and abstract submission, but additional expenses for programming/site design are required.

- *Programs/printing.* Funds are requested for printing of promotional materials, the Conference Program, workshop-specific materials and other essential printing and duplication costs.

- *Fuel.* Funds are requested for diesel for essential support vehicles. The Lassa fever Program maintains two Toyota Land Cruisers (seating 9) and two Toyota Pradas (seating 5). These vehicles will be in service throughout the course of the pre-meeting and the conference, supplementing or bringing attendees to buses, or from hotels. In addition, funds are requested to support additional fuel for generators. Essential Laboratory equipment (freezers and refrigerators) is powered by a solar power system. Additional fuel is needed during the conference to support power to an administrative building where air conditioning and Internet services will be provided.

- *PPE/other workshop supplies.* Visitors to the Lassa Ward and Laboratory and participants in the Ecology Workshop will require Personal Protective Equipment (PPE). This Includes Tyvek, gowns, face masks, eye protection, gloves and shoes covers. All participants will be given a training session in the proper use of the PPE.

- *Childcare and other services.* Funds are requested to supply required child support, family services and other personal services.

- Advertising. Funds are requested to publish an advertisement in a leading open access virology journal.

Additional Sources of Support

Registration fees. A nominal registration fee of $250 will be charged for each participant, not including for “pioneers,” students, African speakers, support and NIAID staff. While the collected registration fees will cover only a minor part of total expenses they will be essential to defray expenses for the meeting not adequately covered under Logistical Support above.

Corporate sponsors. Organizers will follow the guidance of NOT-OD-12-041. Direct charges for meals/food and beverages will not be charged to this project. We expect to obtain a limited amount of additional support from corporate sponsors to help defray nonallowable costs such as for lunches and dinners.

In the event that funds collected from Registration fees and corporate sponsors exceed actual meeting expenses, the additional funded will be used for post hoc increases for African and grad student/postdoc travel awards.

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		0.00
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		0.00
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		86,500.00
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel	86,500.00	
4. Subsistence		
5. Other		
6. Number of Participants/Trainees	65	
Section F, Other Direct Costs		13,500.00
1. Materials and Supplies		
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	13,500.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		100,000.00
Section H, Indirect Costs		0.00
Section I, Total Direct and Indirect Costs (G + H)		100,000.00
Section J, Fee		0.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Prof.
* First Name: Robert
Middle Name: F
* Last Name: Garry
Suffix:

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: Ms.
* First Name: Kathleen
Middle Name: M
* Last Name: Kozar
Suffix:

* Phone Number: 5049885613
Fax Number: 5049881748
Email: elecnotf@tulane.edu

* Title: Director

* Street1: 1430 Tulane Avenue, EP-15
Street2:
* City: New Orleans
County/Parish:
* State: LA: Louisiana
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 70112-2632

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Tracking Number:GRANT11110480 Funding Opportunity Number:PA-10-071 Received Date:2012-04-12T14:12:32-04:00

OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)		Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1240-LW conf grant 4-12-12d	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	1241-LW conf grant 4-12-12d	Add Attachment	Delete Attachment	View Attachment
4. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
5. Progress Report Publication List		Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

6. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table		Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

10. Vertebrate Animals		Add Attachment	Delete Attachment	View Attachment
11. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
12. Multiple PD/PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
13. Consortium/Contractual Arrangements		Add Attachment	Delete Attachment	View Attachment
14. Letters of Support	1242-Lassa Ward Opening Lett	Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)		Add Attachment	Delete Attachment	View Attachment

16. Appendix Add Attachments | Remove Attachments | View Attachments

List of Research Plan Attachments

Opening a New Lassa Fever Ward in Kenema Sierra Leone

Lassa fever (LF) is an acute and often fatal hemorrhagic disease that is endemic in the West African countries of Sierra Leone, Guinea, Liberia and Nigeria. Lassa virus (LASV), a member of the *Arenaviridae*, is the etiologic agent. LF is a zoonotic infection, transmitted to humans by *Mastomys natalensis* (multimammate or bush rat). Recent investigations using newly developed diagnostic assays indicate that overall mortality in primary cases of LF presenting while viremic is over 40%. Mortality rates for LF are over 90% in third trimester pregnancies for both the expectant mother and the fetus. The severity of LF, its ability to be transmitted via aerosol droplets, and the lack of a protective vaccine led to its classification as a NIAID Category A pathogen and biosafety level-4 (BSL-4) agent. The antiviral drug ribavirin has been reported to significantly reduce fatality, but only if it is administered within 6 days after the onset of symptoms.

Kenema Government Hospital (KGH) in Sierra Leone, West Africa is a 350-bed facility situated in the heart of the region with the highest incidence in the world of LF. Because of the importance of LF as a bioterrorism and public health threat, KGH has developed an advanced clinical and laboratory research capacity. Active and passive surveillance for cases of LF is conducted by a KGH team of outreach workers working in collaboration with local health centers. There is an active ecology team at KGH that has decades of experience in trapping rodents and other mammals. The Lassa Research Laboratory, situated on the KGH grounds (completed in 2005) is state-of-the-art. KGH currently maintains a 25-bed Lassa Ward and full-time staff completely dedicated to the care of patients with LF. Recently, the KGH research team has broken ground on a new 48-bed Lassa Ward that will replace this historic, but timeworn facility. This is a request for support of a one-time NIAID conference in association with opening of the new Lassa Ward in Sierra Leone. We will discuss the many recent breakthroughs in our understanding of the virology, immunology, epidemiology and natural history of LF. In this open meeting we hope to bring together experts on LF from several generations of scientists and to cover a broad range of subject matter. We will invite anyone interested to attend, while maintaining uniform standards for selecting the final list of participants. We anticipate that scientists from across West Africa as well as from the United States, South America, Europe, Asia and elsewhere will attend this timely conference.

Specific Aims:

1. Commemorate the opening of the new Lassa Ward, reflect on the historical, societal and scientific impact of LF, and increase public awareness of the continued impact of LF in Sierra Leone and the West African subregion.
2. Assemble several generations of experts on LF who may otherwise not have an opportunity to meet to share and debate their latest findings and to define goals for the future.
3. Present training exercises and workshops to showcase new capabilities in West Africa for LF clinical and laboratory research.
4. Integrate clinical and basic research findings to identify the most critical gaps in our knowledge and understanding of LF and what steps could aid future studies.
5. Promote collaborations to move LF research, including vaccine and therapeutic development, forward at a faster pace. Develop creative solutions to unresolved challenges in research and implementation of LF policies and programs.
6. Provide funds to graduate students, postdoctoral trainees, junior faculty members, and members of under-served and under-represented stakeholder groups for the purpose of attending this conference.
7. Create a setting that will contribute to the probability of success of the conference, and provide adequate support, equipment and other physical resources.

Conference Plan

Kenema Government Hospital (KGH) is situated in the heart of the region with the highest incidence of LF in the world. Because of the importance of LF as a public health threat, KGH has developed an advanced clinical and laboratory research capacity. KGH currently maintains a 25-bed Lassa Ward and full-time staff completely dedicated to the care of patients with LF. Recently, construction has been initiated on a new 48-bed Lassa Ward that will replace this historic, but timeworn facility. Here, we request support for a one-time NIAID conference in association with opening of the new Lassa Ward at KGH (Fig. 1).

Fig. 1. Conference Logo

Justification for the conference (scientific need, timeliness, and usefulness of the conference to the scientific community).

In contrast to many other bioterrorism threats, LF is also a major public health concern. In addition to causing widespread loss of life and social disruption across West Africa, LF is also a serious impediment to economic development because of frequent fatal cases in visiting health care and social workers, and ex-patriots working in fledgling industrial ventures. The challenge of LF to advancement is particularly evident in the emerging pro-West democracy of Sierra Leone. The importance of LF in the field of infectious disease transcends even these facts. Much of the early field research on LF by legendary virologists was based in Sierra Leone. However, the violent civil conflict from 1991 to 2002 (Blood Diamonds War) disrupted virtually all research on LF in Sierra Leone. Immediately following the end of the war a consortium of LF researchers began rebuilding the scientific infrastructure at KGH with support by NIAID, WHO and other partners (vhfc.org). This group has benefitted by the wise council of many of the pioneers in LF research.

For the first time in over four decades sufficient scientific information has accumulated to begin to achieve control of LF in Sierra Leone and the West African subregion. However continued progress in confronting LF requires interdisciplinary collaboration and commitment to science and evidence-based policies. This conference will bring together generations of scientists and stakeholders representing diverse disciplines to tackle the multifaceted challenges of LF. Progress in the fight against LF will require continued scientific advances towards a vaccine, and implementation to scale of successful interventions, including new modern rapid diagnostics. It will require continued investments in human and infrastructure development as exemplified by the new Lassa Ward, and it will require leadership at a global level. The opening of the new Lassa Ward at KGH will have great symbolic importance. We expect that the proposed conference will exert a sustained, powerful influence on the LF research field for at least the next decade.

Objectives

The opening of the new Lassa Ward at KGH presents an opportunity to commemorate decades of basic and clinical research, bring investigators (including the next generation of researchers) who are not familiar with field research to the site, reflect on the impact of a highly significant biodefense and public health pathogen, showcase new scientific advances, promote collaborations, identify gaps in our knowledge and critical barriers to progress in the field, and guide prioritization and implementation of interventions and health policy. In short, the objective of the proposed conference is to guide the way forward for LF research.

Specific Program
The conference is organized to provide challenging content. We will employ several novel approaches and methods to fulfill this purpose. We will cover concepts, approaches, and interventions that are novel to the field of LF research and are also novel in a broad sense. A overview of the format of the conference is provided in Table 1.

Table 1: Opening a New Lassa Fever Ward in Kenema Sierra Leone: Overall Format of the Meeting

	Day 1	Day 2	Day 3	Day 4
morning	Travel; Meeting registration	Workshops and training sessions	State-of-the-art talks/discussions	Round-table discussions
afternoon	Opening Ceremony	Workshops and training sessions continue.	State-of-the-art talks/discussions continue	Travel
evening	Opening Banquet and Keynote addresses	Informal dinners and collaborative sessions	African dinner, poster/collaborative sessions	Departure from Lungi Airport

Specific Aim 1. Commemorate the opening of the new Lassa Ward, reflect on the historical, societal and scientific impact of LF, and increase public awareness of the continued impact of LF in Sierra Leone and the West African subregion.
The opening session (afternoon - day one) of the conference will commemorate the opening of the new Lassa Ward at KGH. The event will feature a dedication of the new facility by the Minister of Health and Sanitation [MoHS] of Sierra Leone. The ceremony will feature brief words from the Director of KGH, the Mayor of Kenema, the US Ambassador to Sierra Leone, and representatives from NIAID, NAVFAC, WHO and other research partners at the site (see Letters). Several of the pioneers in LF research, including Drs. Joe McCormick, Susan Fisher-Hoch, and others, have agreed to reflect on the historical, societal and scientific impact of LF in Keynote addresses to be held on the evening of day 1. The organizers of the conference view this conference as a momentous opportunity to increase public awareness of the continued impact of LF in the West African subregion through invitation of national and international media.

Specific Aim 2. Assemble several generations of experts on LF who may otherwise not have an opportunity to meet to share and debate their latest findings and to define goals for the future.
It is our consistent observation that US or other non-African scientists who visit the LF field site at KGH benefit immensely by the experience. Indeed, it is impossible to not come away from a visit without new perspectives on LF research, including a new appreciation for the environment in which LF occurs and the profound impact of this devastating disease in the West African subregion. This aspect of the conference experience will be particularly valuable for the new generation of scientists working either in BSL-4 or other important research areas that do not directly involve field research. Moreover, many leaders in LF research have not had an opportunity to visit Sierra Leone since the end of the 12-year civil conflict in 2002. This conference will provide a unique opportunity to bring together generations of scientists to reflect on progress, debate future directions and set up new collaborations for LF research.

Specific Aim 3. Present training exercises and workshops to showcase new capabilities in West Africa for LF clinical and laboratory research.
Day two of the conference will be dedicated to innovative training exercises and workshops, all of which will be presented and managed by Sierra Leonean experts (see also RESOURCES). For orientation of the days activities Dr. Donald S. Grant, Director of the LF Program at KGH,

will present an overview of operations at KGH with an emphasis on clinical management of LF from 8:30 – 9:30am. The following workshops and training exercises will then be available and repeated at various times as needed for all attendees:

The New Ward. *Organizers/presenters: Dr. Donald S. Grant, Director of LF Program, Chief Clinician Lassa Ward and Mrs. Balu Fonnie, Head Nurse Lassa Ward.* All conference participants will have the opportunity to tour the new Lassa Ward. Appropriate Personal Protective Equipment (PPE) and training in its use will be provided. Tours will be limited to groups of about 10-15 participants at a time, with as many scheduled tours as necessary to accommodate all participants. Following the tour the groups will engage in a discussion session.

An ongoing series of other tours or workshops (repeated as necessary) will interdigitate with the Lassa Ward tours, as follows:
The Lassa Research Laboratory. *Organizers/presenters: Mr. Augustine Goba, Head of the Lassa Laboratory and Technicians: Mohammed Fullah, Mambo Mamou and Bokarie Bayone.* Lassa laboratory capabilities, including the new generation immunodiagnostics (rapid tests), with highlight opportunities for collaboration and capacity building (see Resources). Participants will be provided with appropriate PPE and training to enter the Lassa Laboratory in groups of 6-8. A discussion session will to follow to debate challenges for LF diagnostics, particularly the rollout of such assays across West Africa

Meetings with Lassa Ward Nurses and LF survivors in the Old Lassa Ward
Organizers/presenters: Ms. Veronica Koroma (Nurse, Lassa Ward and herself a LF survivor). Participants will tour the decommissioned old Lassa Ward and meet with 5-6 LF survivors. There will be a Q&A with Mende and Krio translators provided.

The afternoon of day two will showcase opportunities for fieldwork in Kenema district. Not all participants will participate in each of these opportunities, but will be asked to prioritize which they wish to participate in.

Outreach and sensitization workshop. *Organizers/presenters: Richard Fonnie and Lansana Kanneh.* Approximately 12-16 non Sierra Leoneans participants [Sierra Leoneans will be provided this opportunity at other dates] divided into two separate teams (6-8) will have an opportunity to go out with members of the highly experienced outreach team to participate in a typical session with villagers. The outreach team provides a wealth of information on important prevention and control measures, routes of transmission, and the signs and symptoms to watch for in those infected with LASV (see Resources). Community outreach also aims to inform potential study participants on studies being conducted, the risks and benefits of participation, confidentiality, and the procedures and expectations of those that decide to participate.

Visits to field sites at Panguma, Seguema and Gondama. *Organizers/presenters: Mohamed Sow and Mohamed Fomgbeh.* The Panguma Mission Hospital and Nixon Hospital at Seguema are historic sites for LF research, with many of the original studies on LF in Sierra Leone having been conducted at these locations (see Resources). These sites, as well as the Gondama Hospital (affiliated with *Medecins Sans Frontieres*) refer large numbers of LF Patients to KGH. This afternoon workshop will be appropriate for participants with disabilities. Two air-conditioned buses operating on a staggered schedule will accommodate up to 80 participants.

Rodent trapping at LF case houses. *Organizers/presenters: Willie Roberts, Kandeh Karbo, James Koninga.* Transmission of LF occurs via contact with urine, feces, and saliva of *Mastomys natalensis*. Approximately 10-12 non Sierra Leoneans participants divided into two

teams (5-6) will have an opportunity to go out with the highly experienced ecology team to trap rodents at case houses in two villages (see Resources). Sierra Leoneans will be provided this opportunity at other dates. Staying overnight in the villages is the norm. Because the meeting will be held in the "dry season" weather will likely not be an issue. Tents are used as staying in village homes adds additional risks of exposure to LASV. The participants will be provided with tents, PPE and other gear essential for this activity, and will return early enough to fully participate in the day 3 activities. If needed, an "overflow" workshop will be held on Day 3.

Rounding in the Lassa and General Wards. *Organizers/presenters: Drs. Donald S. Grant, Humarr Khan or S. B. Sesay.* Approximately 12-15 participants divided into three separate teams (5-6) will have an opportunity to round in the Lassa Ward and general KGH wards with Drs. Grant, Khan or Sesay. This opportunity will be limited to participants with Medical Degrees. General tours of KGH albeit without direct patient contact will also be available.

Table 2. State-of-the-art in LF research (tentative schedule)	
Epidemiology of LF in Sierra Leone and beyond	Margaret Lamunu – WHO (invited)
Structural Biology of Arenaviruses	Erica Ollmann-Saphire - Scripps (confirmed)
Arenavirus Biology	Jack Nunberg – U Montana (confirmed)
Natural History of Lassa fever and clinical management of patients	Donald Grant; Balu Fonnie, Veronica Koroma – KGH (confirmed)
Ecobiology of the rodent reservoir of Lassa fever	Elisabeth Fichet-Calvet - Bernhard Nocht Institute Hamburg (invited)
Immunopathology of Lassa fever	Yoshi Kawaoka - U Tokyo/U Wisc. (invited), Michael Buchmeier (UC Irvine) (confirmed)
Lassa fever diagnostics	Luis Branco - Zalgen (confirmed), Augustine Goba (KGH) (confirmed)
Animal models of Lassa fever	Tom and Joan Geisbert – UTMB (confirmed)
Lessons from other arenaviruses	Delia Enria - Argentina (invited); Michael Oldstone (Scripps) (confirmed)
Lassa fever vaccine candidates	Maria Salvato - U Maryland (confirmed)
Viral, human and rodent genetics of Lassa fever	Pardis Sabeti - Harvard/Broad Institute (confirmed)
Antiviral drugs for Lassa fever: beyond ribavirin	Dennis Hruby or Sean Amberg – Siga Techologies (confirmed)

Specific Aim 4. Integrate clinical and basic research findings to identify the most critical gaps in our knowledge and understanding of LF and what steps could aid future studies. The third day of the conference experts in LF will present 15-30 minute talks specifically to identify the most critical gaps in our knowledge and understanding of LF and what steps could aid future studies (Table 2). The emphasis will be on unpublished state-of-the-art research. We expect that the training exercises and workshops will serve to energize the Day 3 sessions. Additional topics for the state-of-art lectures will be selected from the abstracts.

Specific Aim 5. Promote collaborations to move LF research, including vaccine and therapeutic development, forward at a faster pace. Develop creative solutions to unresolved challenges in research and implementation of LF policies and programs. The final session of the conference (morning of day 4) of the conference will be dedicated to panel (round-table) discussions on the following topics (tentative schedule - Discussion Leaders in parentheses will begin with 15 minute overviews):

- Structural Biology of Arenaviruses, Kate Hastie – Scripps (confirmed)
- Immunopathology of Lassa fever, James Robinson – Tulane (confirmed), Stefan Kunz – U Lausanne (confirmed)
- Public Health interventions for Lassa fever, Alhassan L. Seisay – SL MOHS (confirmed), Melissa Leach - U Sussex (confirmed)
- Lassa fever vaccine and therapeutic candidates, Connie Schmaljohn –USAMRIID (confirmed)

Additional topics for panel discussions will be selected from the abstracts.

Specific Aim 6. Provide funds to graduate students, postdoctoral trainees, junior faculty members, and members of under-served and under-represented stakeholder groups for the purpose of attending this conference.
If this application is successful, the conference will provide funds for travel awards to senior virologists (speakers) and junior scientists. Availability of awards will be publicized as described below before the deadline for early registration. To be eligible for an award, the applicant must (with some exceptions) be either an invited speaker, a graduate or post-doctoral student, or a new, junior (untenured) faculty member, as well as the first author of an abstract. Travel funds will be awarded based on financial need and underrepresented minority status.

Specific Aim 7. Create a setting that will contribute to the probability of success of the conference, and provide adequate support, equipment and other physical resources.
Expected size and composition of the audience, and method of selection. In this open meeting we will bring together experts on LF to cover a broad range of subject matter. We will invite anyone interested to attend, while maintaining uniform standards for selecting the final list of participants. We will limit the size of the audience to approximately 120 people. This number is based on number of hotel rooms in Kenema and human and physical resources available to manage transport of conference attendees. The composition of the audience will be diverse mix of Americans, Europeans, Asians and Africans. Approximately 60% Africans, African-Americans or other “minorities.” 50% of presenters and workshop leaders shall be female. The goal is to balance scientific and public health expertise as well, although the majority (approximately 70%) of presenters will represent bench scientists or clinicians. We also seek a balanced mixture of scientists and other representatives from multiple generations of LF investigators.

Composition and role of the organizing committee. Robert Garry (Tulane University) will chair the Organizing Committee. Additional members of the Organizing Committee are Pardis Sabeti (Harvard/Broad), Maria Salvato (IHV, U Maryland) , Tom Geisbert (UTMB), Connie Schmaljohn (USAMRIID), Joseph McCormick (UTx Houston) and Juan C. de la Torre (Scripps). Letters from these individuals are attached. Each individual is a leader in the field of LF research. The Viral Hemorrhagic Fever Consortium (vhfc.org) has assumed responsibility for the administrative and financial management of the meeting. Registration documents and abstracts will be submitted electronically to a secure website at vhfc.org for 24 hour access to online registration and payment. The top abstracts will be chosen for oral presentations, and remaining scientifically acceptable abstracts will be assigned to the poster session.

Involvement of women, minorities, and persons with disabilities, and resources for childcare and other types of family care. The conference has been carefully planned for the appropriate involvement of women, minorities, and persons with disabilities. Individuals from all these groups were involved in planning or will participate in the conference. Invited speakers/round-table leaders (day 3) are >50% female, which significant minority representation. 5 continents are represented thus far. Temporary or permanent unilateral or bilateral deafness occurs in ~30% of LF patients. The workshop with LF survivors shall include those with

permanent unilateral or bilateral deafness acquired during LF. Child and family care will be provided by experienced caregivers at the Tulane Project House, which is a short distance from the conference venues.

Publicity. The meeting will be advertised directly by email to all research groups that have actively publishing in the scientific literature on VHF over the past decade. An advertisement on the ”front page” of *Virology Journal*, a leading open access journal in virology, will be read by basic biomedical scientists outside the VHF community is planned to promote awareness of the meeting to new prospects, especially those outside of North America and Europe. The proceedings of the conference will be published in *Virology Journal.*

Related conferences/meetings held on the subject during the past 3 years. There are a number of conferences held each year on biodefense pathogens. An example is the ASM Biodefense conference held in Washington DC. There are also conferences held on specific biodefense pathogens, the most prominent of which is the Filovirus Conference last held in Tokyo, Japan. None of these conferences are specifically dedicated to LF. Recently, researchers from the West African subregion gathered in Freetown Sierra Leone for a very productive meeting on LF sponsored by WHO. However, basic and clinical research topics were not discussed in depth, rather the focus was almost exclusively on health policy. The current meeting seeks to expand and better connect the global base of researchers engaged in LF basic and clinical research.

Potential problems and alternative strategies. Holding an international conference in West Africa presents several potential problems that the conference organizers and the team at KGH are well prepared to address. The airport in Lungi is distant from Freetown, which in turn is a 4.5 hr transit from KGH. Several alternatives are available for transport. Sierra Leone has recently acquired a new fleet of air-conditioned modern buses, two of which will be leased for this meeting. Accommodations in Kenema are limited, which is why participation will be limited to 120. A new conference center being constructed near the city council building will be completed in May, 2012 (see Resources). Access to the Internet is limited in Sierra Leone, but a SatCom system is operational at KGH and will be made available to participants in the Lassa Laboratory Library. The task of organizing field workshops to remote villages in SL can be considered daunting. However, KGH personnel make these visits every week of the year and are often accompanied by nonSierra Leonean visitors. The temperature in February to March can be hot (85->100^{o}F), but the rainy season does not begin until about May or June. Participants will be continuously encouraged to properly hydrate. Potential exposure of participants to LASV will be minimized and no participant will enter the Lassa Laboratory or Ward without giving informed consent. All participants will be given a training session in the proper use of PPE.

Benchmarks for success. We will consider the conference a success if the following benchmarks are achieved:

- Successful dedication of the new LW with articles in both local press and international media.
- Participation of 120 individuals with over 50% “minorities” and >40% women.
- Participation of 100% of attendees in 5 or more workshops or training exercises.
- Initiation of >5 new collaborations in LF research, including vaccine and therapeutic development.
- Publication of a summary of the meeting in an open access journal elaborating on proposed creative solutions to unresolved challenges in research and implementation of LF policies and programs.

none

Letters of Support for Opening of New Lassa Ward Conference

1. Letter from NIAID
   Katrin Eichelberg, PhD

2. Letter from United States Embassy in Sierra Leone
   Mitchell P. Benedict, Chargé d' Affairs, a.i.

3. Letter from Mayor of Kenema
   Chief Brima Kargbo

4. Letters from Ministry of Health and Sanitation of Sierra Leone
   Dr. S.B. Seisay
   Dr. Donald S. Grant

5. Letters from Organizing Committee and other supporters
   Juan C. de la Torre, MD
   Thomas W. Geisbert, PhD
   Dennis Hruby, PhD
   Stefan Kunz, PhD
   Igor Lukashevich, MD, PhD
   Joseph B. McCormick, MD
   Jack Nunberg, PhD
   Michael B.A. Oldstone, MD
   Pardis Sabeti, MD, DPhil
   Maria Salvato, PhD
   Connie S. Schmaljohn, PhD

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892

March 13th, 2012

Robert F. Garry, PhD
Professor/Assistant Dean
Department of Microbiology and Immunology
Tulane University School of Medicine
1430 Tulane Avenue
New Orleans, Louisiana USA 70112

Dear Dr. Garry:

The National Institute of Allergy and Infectious Diseases has reviewed your request for an application for a conference grant (R13) entitled "Opening of the New Lassa Ward in Sierra Leone" and determined that it will accept it for primary assignment and peer review. Please be advised that funding of your application will depend on the outcome of the review, NIAID priorities, and the availability of funds.

Sincerely,

Katrin Eichelberg, Ph.D.
Office of Special Populations and Research Training
Division of Extramural Activities
National Institute of Allergy and Infectious Diseases

Embassy of the United States of America

Freetown, Sierra Leone
April 11, 2012

Robert F. Garry, Ph.D.
Tulane University School of Medicine
1430 Tulane Avenue
New Orleans, LA 70112

Dear Professor Garry:

Thank you for your recent presentation on Lassa fever at the U. S. Embassy. We appreciated very much the update on the scientific research and the capacity building your team has done in Kenema. The potential for improved diagnostics and a vaccine is exciting.

My colleagues and I were also pleased to learn about progress toward a new Lassa ward in Kenema. The facility in Kenema is important to Lassa fever research in all of West Africa, and the dedication of you and your colleagues has led to its recognition as a center of excellence for research on this critical infectious disease.

The opening of the Lassa ward next year will be a wonderful opportunity to bring together doctors, scientists, outreach staff, lab technicians, and government workers from both Sierra Leone and the United States to continue to share knowledge and build a deeper understanding of this problem.

The U.S. Embassy strongly supports the concept of utilizing the occasion of the opening of the new Lassa ward in Kenema to engage the many people who have dedicated their lives to understanding this disease in a conference. The U.S. Embassy will look forward to learning more about this conference and we believe it will be an important event for Lassa fever and for Sierra Leone.

Sincerely,

Mitchell P. Benedict
Chargé d'Affaires, a.i.

April 2 2012
The Mayor
Kenema City Council
Kenema
Sierra Leone

Dear Professor Garry,

I was pleased to hear about the recent approval to begin construction on the Lassa ward in Kenema Government Hospital. As Mayor of Kenema, I think this is a wonderful development not just for the hospital, but for Kenema and the surrounding regions. Kenema will welcome an opening ceremony to celebrate such an event and will look forward to hosting the many people who have contributed to the research of Lassa fever.

I think the conference will provide a good opportunity to introduce the lovely town of Kenema to your colleagues and serve as a perfect backdrop for Sierra Leoneans who have spent their lives researching Lassa to meet Americans who have done likewise.

I look forward to being involved in helping to plan how our great city will receive the members of your conference.

Sincerely,

MAYOR KENEMA CITY COUNCIL DATE SIGN

Chief Brima Kargbo

GOVERNMENT OF SIERRA LEONE
MINISTRY OF HEALTH AND SANITATION
KENEMA GOVERNMENT HOSPITAL

FROM: The Medical Superintendent
Kenema Government Hospital

TO: Professor Garry

April 2, 2012

Dear Professor Garry,

I have been excited to hear about the progress of our Lassa ward and think it is an event worthy of commemorating. Even more important, though, will be the opportunity to showcase how far we have come in our understanding of Lassa fever over the years.

The relationship between Tulane University and Kenema Government Hospital has produced many important findings. When all the collaborators who have contributed to this research are gathered for the opening of the Lassa ward, it will be a welcome opportunity to review how far we have come. Also, it will be a good time to consider how far we have left to go in order to beat this infectious disease.

I support this idea of making this conference more interactive, engaging and useful to this community. Let us work together to make this happen.

Sincerely,

Dr S.B. Seisay

GOVERNMENT OF SIERRA LEONE
MINISTRY OF HEALTH AND SANITATION
THE LASSA FEVER ISOLATION UNIT
KENEMA GOVERNMENT HOSPITAL

April 2, 2012

Dear Professor Garry,

As Director of the Lassa fever program in Kenema Government Hospital, I see the upcoming completion of the Lassa ward as a seminal event in the history of the collaborative fight against this disease. I have spent much time with patients and family members of patients and know how important this new ward will be to them in particular.

I think the suggestion to create an innovative conference around the opening of the ward is a good and necessary thing. We have made progress against Lassa fever but much remains to be done. It will be critical to use this opportunity to not only celebrate our latest achievement, but to brainstorm about the next big break in the science which surrounds this disease. Transmission, efficacy of ribavirin—these are all remain open and important questions. Let us use this opportunity to gather the best minds in the field to start tackling the new challenges in Lassa.

I look forward to working together with you closely on making this conference one that might contribute deeply to our understanding of Lassa fever

Sincerely,

Dr. Donald S. Grant

THE SCRIPPS RESEARCH INSTITUTE®

Juan C. de la Torre, Ph.D.
Professor
Department of Immunology and Microbial Science

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March 5, 2012

Robert F. Garry, Ph.D.
Professor/Assistant Dean
Department of Microbiology and Immunology
Graduate Program in Biomedical Sciences
Tulane University School of Medicine
1430 Tulane Avenue
New Orleans, Louisiana USA 70112

Dear Robert,

This letter is to express my support and enthusiasm for your proposal to organize a conference in association with the opening of the new Lassa Ward in early to mid 2013 at the Kenema Government Hospital (KGH) in Sierra Leone to discuss recent breakthroughs in our understanding of the virology, immunology, genetics (viral, rodent, human), epidemiology, pathobiology and natural history of Lassa fever.
I wish you success with your application for NIAID support for this timely and very important conference.

With all best wishes,

Juan C. de la Torre

UTMB
The University of Texas Medical Branch

SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY

7 March 2012

Robert F. Garry, PhD
Professor/Assistant Dean
Department of Microbiology and immunology
Graduate Program in Biomedical Sciences
Tulane University School of Medicine
1430 Tulane Avenue
New Orleans, Louisiana USA 70112

Dear Bob,

I am writing to offer my enthusiastic support for your initiative to organize a conference in association with the opening of the new Kenema Government Hospital Lassa Ward to discuss the many recent breakthroughs in the fields of virology, immunology, genetics, epidemiology, pathobiology and natural history of Lassa fever. This is an extremely important and highly overlooked public health problem. Your efforts to combat Lassa virus and draw attention and resources to West Africa is appropriate and admirable.

Bringing together experts in Lassa fever from various parts of the world to discuss the challenges of managing Lassa virus infection in West Africa is an important step in beginning to tackle issues including the development and implementation of effective countermeasures. I wish you all the best in this endeavor and am happy and available to assist in this effort.

Sincerely,

Thomas W. Geisbert, PhD
Professor, Microbiology and Immunology
University of Texas Medical Branch
Galveston National Laboratory
301 University Blvd.
Galveston, TX 77550-0610
Phone: 409-266-6906 Fax: 409-266-6810
Email: twgeisbe@utmb.edu

SIGA
Human BioArmor™

Robert F. Garry, Ph.D.
Department of Microbiology and Immunology
Tulane University School of Medicine
1430 Tulane Avenue
New Orleans, LA 70112

Dr. Garry-

I am writing this letter to express my support for your efforts in organizing a conference dedicated to discussing the current state of our understanding of Lassa fever. As you know, the drug discovery efforts here at SIGA Technologies have been focused on biodefense and emerging pathogen targets. We have had an active and ongoing program to discover and develop preclinical candidates for treating arenaviruses, and Lassa fever is one of our principal targets. We recognize and appreciate your efforts as the Program Manager of the Viral Hemorrhagic Fever Consortium. The infrastructure you're developing may well be instrumental in the clinical development of new therapeutics. We think that a meeting of Lassa experts spanning multiple disciplines is an excellent idea, and we enthusiastically encourage your endeavors.

Sincerely,

Dennis E Hruby

Dennis E. Hruby, Ph.D.
Chief Scientific Officer

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Robert F. Garry, PhD
Professor/Assistant Dean
Department of Microbiology and Immunology
Graduate Program in Biomedical Sciences
Tulane University School of Medicine
1430 Tulane Avenue
New Orleans, Louisiana 70112

Lausanne, March 14. 2012

Dear Robert.

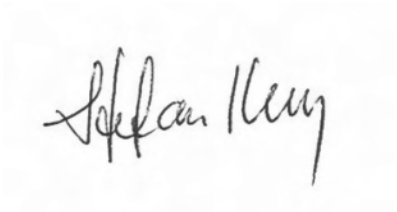
It is a great pleasure to provide a letter of support for your planned NIAID conference associated with the opening of the new Lassa ward of the Kenema Government Hospital in Sierra Leone in 2013.

While most viral hemorrhagic fevers are relatively rare diseases associated with sporadic outbreaks, Lassa fever is an endemic disease of large proportions causing several hundred thousand infections per year with thousands of deaths. Considering the large number of people affected and the severe humanitarian impact, Lassa fever is arguably among the most neglected tropical diseases. The opening of the new Lassa ward and your planned conference associated with it are very positive signals that will enhance the awareness of Lassa fever as a major disease in this region of the world.

Your planned conference in an affected region will attract clinicians and researchers working on different aspects of Lassa fever, including basic virology of Lassa virus, epidemiology, viral immunobiology, viral pathogenesis, and development of novel diagnostics, therapeutics, and vaccines. Unique in its scope and setting, such a conference would promote interactions between experts representing very different aspects of Lassa fever research. The exchange of ideas will create synergisms and lead to fruitful new collaborations that will help to further advance the field.

My laboratory in fundamental virology has a long-standing interest in arenaviruses and in particular Lassa virus. Our work combines basic research on Lassa virus-host cell interactions with translational efforts aiming at the development of novel therapeutics against this important human pathogen. The development of local clinical infrastructure dedicated to Lassa fever is pivotal for the future implementation of the results of our basic and translational research on Lassa virus. Your initiative is therefore of utmost importance for us in basic research and I support it with enthusiasm.

Sincerely Yours



Stefan Kunz

UNIVERSITY OF
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CENTER FOR
PREDICTIVE MEDICINE

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School of Medicine, Department of Pharmacology and Toxicology
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Robert F. Garry, PhD
Professor/Assistant Dean
Department of Microbiology and immunology
Graduate Program in Biomedical Sciences
Tulane University School of Medicine
1430 Tulane Avenue
New Orleans, Louisiana USA 70112

March 6, 2012

Dear Robert,

I am delighted to write a letter in support of your initiative to organize an NIAID conference in association with opening of new Lassa Ward in Sierra Leone. The reality is that among all NIAID Category A Priority Pathogens, the only Lassa virus is responsible for thousands of deaths annually in West Africa. For decades this problem was underestimated and Lassa Fever researches have suffered from lack of funding. Fortunately, during last years the situation changed. The opening of new Lassa Ward will prove this positive trend. It is a great idea to build-up on this momentum and to bring together experts on LF from several areas of expertise to better understand the virology, immunology, genetics, epidemiology, pathobiology, natural history of the disease, and to design therapeutic and preventive strategies.

Vaccination is the most effective and practical measure to control LF in West Africa. For last 20 years my lab was involved in the development of LF vaccines. With NIAID support we have a few vaccine candidates in pre-clinical development. These vaccine candidates can potentially target different groups of the population at risk. Development of the diagnostic and clinical infrastructure and capabilities in endemic areas is an extremely important for future vaccine studies in the field. I am confident that this time will come, sooner or later.

Sincerely Yours,

Igor

Igor S. Lukashevich, MD, PhD
Professor and University Scholar

Clinical and Translational Research Building • 6th Floor • 505 South Hancock St. • Louisville, KY 40202
P: 502.852.5586 **F:** 502.852.5468

UTHealth™
The University of Texas
Health Science Center at Houston

School of Public Health
Brownsville Regional Campus

April 4, 2012

Dr. Robert Gary,

Organizing Committee

NIAID Conference: "Opening a New Lassa Fever Ward in Kenema Sierra Leone"

Dear Bob,

It is with great pleasure and not a little nostalgia that I register my enthusiastic support for the proposed NIAID conference to commemorate the opening of a new Lassa fever research program and clinical ward in Kenema, Sierra Leone.

As you are well aware I was responsible for establishing the Lassa Fever Research Program in Sierra Leone in 1976 and the program continued until the war started in 1991. I have had the opportunity to return some years ago to meet with many of the original Lassa team at Segbwema Hospital. Indeed the original laboratory (picture attached) was still there, albeit inactive. During that time much new information was learned about Lassa by many who worked on the project. The technology of today provides unparalleled opportunity to characterize at the molecular level, the clinical and broader observations made by many over the ensuing years. Furthermore, the future of a vaccine looks very bright and I can think of no better place to develop the field site for trials than this area of Sierra Leone. Thus the opportunity to bring together scientists working on Lassa virus and other arenaviruses to Kenema is very timely. It will also be very useful for scientists working on this important disease to directly observe the clinical and basic science research infrastructure that has been built at Kenema Government Hospital which will hopefully create opportunities for new productive collaborations.

Please keep me informed of the plans for this groundbreaking conference. I would very much like to participate if at all possible, if nothing more than to add a historical perspective on this virulent but fascinating infection. I can be contacted at 956-882-5165 or via email at Joseph.B.McCormick@uth.tmc.edu.

Sincerely,

Joe

Joseph B. McCormick, MD
Regional Dean and James H. Steele Professor

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Brownsville, Texas 78520

The University of Montana

Montana Biotechnology Center
Missoula, Montana 59812

Jack Nunberg, Ph.D.
Director and Professor
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Robert F. Garry, Ph.D.

March 8, 2012

Tulane University School of Medicine

1430 Tulane Avenue

New Orleans, LA 70112

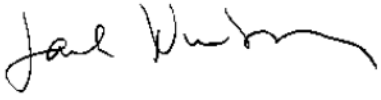
Dear Bob,

Thank you for informing the research community of the important work that is taking place in Sierra Leone. I agree that support embodied in the international symposium you propose would bolster visibility for the project in Africa, and highlight the contributions of the US government and NIAID.

In addition, I would argue that the clinical and community services of the Kenema Government Hospital will be essential to US researchers and pharmaceutical companies as we plan a path to FDA licensure for the many vaccines and antiviral agents currently under development as part of the US biodefense program. It is becoming increasingly clear that animal models will not be sufficient and that clinical studies will be required. Support for KGH is an investment towards the prevention and treatment of Lassa hemorrhagic fever, and may offer additional dividends in addressing arenavirus hemorrhagic fevers in this hemisphere.

Good luck in your efforts to organize this valuable conference. Please keep me informed as to your progress. I look forward to attending and contributing to the development of this important public health and clinical research facility.

Sincerely,



Jack Nunberg, Ph.D.

THE SCRIPPS RESEARCH INSTITUTE®

Michael B.A. Oldstone, M.D.
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Viral-Immunobiology Laboratory
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March 6, 2012

Robert F. Garry, Ph.D.
Professor/Assistant Dean
Dept. of Microbiology and Immunology
Tulane University School of Medicine
1430 Tulane Avenue
New Orleans, LA 70112
rfgarry@tulane.edu

Dear Bob:

As you know I have spent my scientific career in study of the pathogenesis of Old World arenaviruses. Over the last several years we have increased our focus on Lassa fever virus (LASV) using the knowledge we have gleaned from lymphocytic choriomeningitis virus to apply to LASV.

Currently we are intrigued by and evaluating the genome-wide screen from the endemic LASV infection areas that have found the host gene under the greatest positive selection being mutations in the glycosyltransferase enzyme LARGE. We earlier defined LARGE as being essential for the functional maturation of the host cell receptor for LASV, alpha-dystroglycan (α-DG). You and I have had several conversations of utilizing your expertise and the facilities you have been involved with in Sierra Leone LASV endemic area for the procurement of samples from patients in the clinic to both further map the mutations in LARGE and to define biologically the importance of such mutations in LASV pathogenesis. Our ultimate goal is the design of therapeutic agents to control LASV infection and develop a genetic screen to identify the 10% of those infected with LASV who will succumb to the disease.

Your planning of a symposia in Sierra Leone to bring in the many interested and committed players studying LASV is very timely and necessary. The exchange of information should lead to working groups who will strive to tame this infection, exchange ideas, and uncover knowledge.

I heartily support your efforts and strongly urge the NIH to plan and fund such a meeting.

Best regards,

Michael

Michael B.A. Oldstone, M.D.
Head, Viral-Immunobiology Laboratory

MBAO/glw

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April 4, 2012

Dear Dr. Garry,

It is my great pleasure to support you in your proposal for an NIAID conference commemorating the opening of the new ward for Lassa fever patients at the Kenema Government Hospital in Sierra Leone. I have had a longstanding and very productive collaboration with you and your outstanding team at Tulane University working on this devastating and often overlooked disease, and I believe that this conference has great potential to advance Lassa fever research.

Together with our collaborators on-site at KGH, I have had the great benefit to work on the project alongside you, using the exceptional resources and infrastructure we have developed to carry out research in West Africa. We have collected nearly 1000 samples over the last year, which include collections from patients with Lassa fever and other febrile illnesses, and large-scale community collections of individuals to serve as controls for our research and many other future studies.

I believe that your proposal for an NIAID conference is timely and important. Research on Lassa fever has been progressing tremendously in the past few years, and the opening of a new ward at KGH, the regional center of excellence for LF treatment and diagnosis, is a perfect opportunity to bring together leaders in the field. I anticipate that such a conference will give rise to productive research collaborations, and will also allow scientists and medical staff in this field to observe and learn from the clinical and research infrastructure that we have worked to establish.

I wish you the very best with your proposal and look forward to our continued work together.

Sincerely,

Senior Associate Member
Infectious Disease Initiative
Broad Institute

INSTITUTE OF HUMAN VIROLOGY
at the University of Maryland School of Medicine

Robert F. Garry, PhD
Professor/Assistant Dean, Dept of Microbiology and Immunology
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office:504-988-2027; lab: 504-988-3818; mobile: Personal Info
fax: 504-988-1994; email: rfgarry@tulane.edu; web: virology.net; journal: virologyj.com

Dear Dr. Garry,

I enthusiastically support your efforts to organize a conference marking the opening of the new Kenema Government Hospital Lassa Ward in 2013. This historic conference should re-unite Lassa experts who have been working over the last 25 years, and will be a chance to discuss the many breakthroughs in our understanding of the virology, immunology, viral genetics, host genetics, epidemiology, pathobiology and natural history of Lassa Fever.

My own research on Lassa Fever began with my training as an RNA sequencer and molecular geneticists with Fred Sanger and Sydney Brenner at the MRC Laboratories in Cambridge, England. I applied my expertise to complete the sequence of LCMV that had been started by David Bishop's group and by Peter Southern in Mike Oldstone's group. After 5 years in Mike Oldstone's laboratory I moved on to a faculty job at Madison, Wisconsin where, with the help of Igor Lukashevich, Mahmoud Djavani and the CDC team of Stuart Nichol and C. J. Peters, it was possible to also complete the Lassa genomic sequence. The unusually long 3' non-coding region on the Lassa L RNA was noted then and has recently been shown to contribute to its pathogenicity. Dr. Lukashevich and myself have safety-tested an attenuated Lassa Fever vaccine, ML29, which was offered to the Minister of Health in Nigeria by Robert C. Gallo in 2004. Unfortunately, that country did not have the resources to make a GMP product of ML29. ML29 could also serve as a treatment since it is capable of averting disease in animal models when given two days after Lassa exposure. We have since turned our attention to making non-infectious Lassa vaccines.

Thank you for creating this golden opportunity to unite with other researchers and public health authorities to solve the public health problems in a Lassa Fever endemic area.

Sincerely,

Maria Salvato

Maria Salvato, PhD
Professor, Inst of Human Virology, Univ of MD School of Medicine
Associate Director of Global Virus Network for IHV
Baltimore, MD 21201
Office 410-706-1368; FAX 410-706-5198; email: msalvato@ihv.umaryland.edu

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES
1425 PORTER STREET
FORT DETRICK, MARYLAND 21702-5011

March 8, 2012

REPLY TO ATTENTION OF: Office of the Chief Scientist

Robert F. Garry, Ph.D.
Professor/Assistant Dean
Graduate Program in Biomedical Sciences
Tulane University School of Medicine
1430 Tulane Avenue
New Orleans, LA 70112

Dear Dr. Garry,

I am writing in support of your plan to organize an NIAID conference on Lassa fever to be held in Sierra Leone in conjunction with the opening of the new Lassa Ward that you are building there. The impact of Lassa virus on human health in this region of the world is greatly underappreciated, and the conference that you propose would help to focus attention on this problem. In addition, such a conference would promote interactions among scientists from around the globe who can contribute to developing effective diagnostics, vaccines and therapeutics for treating Lassa fever.

In short, I applaud your effort to improve the health of people afflicted with Lassa virus infections and I enthusiastically support your efforts to bring together a community of scientists interested in helping you with this public health problem.

Please let me know if I can be of further assistance and good luck!

Sincerely,

Connie S. Schmaljohn, Ph.D.
Senior Research Scientist for Medical Defenses Against Infectious Diseases (ST)
Office of the Chief Scientist

PHS 398 Checklist

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☒ Yes ☐ No