From: "David J Wolking" <djwolking@ucdavis.edu>
Sent: 07/06/2017 3:06:46 PM (-07:00)
To: "Ricardo Echalar" <rechalar@usaid.gov>
Cc: "Jonna Mazet" <jkmazet@ucdavis.edu>; "PREDICTMGT" <predictmgt@usaid.gov>; "Sarah Paige" <spaige@usaid.gov>; "Richard Greene" <rgreene@usaid.gov>; "Dennis Carroll" <dcarroll@usaid.gov>
Subject: Re: AORs/TAs ACTION REQUIRED Fwd: REQUEST FOR MATERIALS: GHSA Toolkit
Attachments: Field sampling.zip

Hi again,

Here is part 2 of 2 with PREDICT's field sampling protocols and guides.

Cheers,

David

On Thu, Jul 6, 2017 at 3:05 PM, David J Wolking <djwolking@ucdavis.edu> wrote:

> Ricardo,
>
> It is our pleasure. We hope these are useful for the repository and please let us know if you or Casey and the CDC team have any questions or need clarification.
>
> I'm attaching part 1 of 2 here, these are all zipped files by topic, included here are PREDICT tools or guides/protocols on:
>
> - Disease modeling and analytics
> - One Health case studies and lessons learned
> - Guides for behavioral risk investigations
> - Biosafety and lab safety protocols
> - Safe sample transport and shipping
> - A few of our Emerging Disease Insights (designed to inform policy and zoonotic disease surveillance)
>
> Part 2 coming next will be our field sampling guides and protocols.
>
> David
>
> On Thu, Jul 6, 2017 at 2:45 PM, Ricardo Echalar <rechalar@usaid.gov> wrote:
>
>> Eek. I think breaking it up in pieces. Thanks for your help!
>>
>> Ricardo
>>
>> – –
>>
>> Ricardo Echalar, MPH
>> Senior Public Health Advisor
>> Office of Infectious Diseases, Emerging Threats Division
>> Bureau for Global Health
>> U.S. Agency for International Development (USAID)
>> 1300 Pennsylvania Ave, NW, 3.6-53, 3rd Floor, RRB
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>> (M) REDACTED | (W) +1.202.712.4003 | E-mail: rechalar@usaid.gov

On Thu, Jul 6, 2017 at 5:43 PM, David J Wolking <djwolking@ucdavis.edu> wrote:
Hi Ricardo,

I've been working on this today with our operations team leads and have a rather large file (>35MB) prepared. What's the best way to get it to you? Break it up into pieces or share to a Drive link?

Thanks!

David

On Thu, Jul 6, 2017 at 2:29 PM, Ricardo Echalar <rechalar@usaid.gov> wrote:
Hi, David,

I got an out of office response from Jonna. Could you or someone else from the PREDICT team help with this request? Thanks,

Ricardo

– –

Ricardo Echalar, MPH
Senior Public Health Advisor
Office of Infectious Diseases, Emerging Threats Division
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---------- Forwarded message ----------
From: **Ricardo Echalar** <rechalar@usaid.gov>
Date: Thu, Jul 6, 2017 at 5:26 PM
Subject: Fwd: AORs/TAs ACTION REQUIRED Fwd: REQUEST FOR MATERIALS: GHSA Toolkit
To: Jonna Mazet <jkmazet@ucdavis.edu>
Cc: PREDICTMGT <predictmgt@usaid.gov>, Sarah Paige <spaige@usaid.gov>, Richard Greene <rgreene@usaid.gov>, Dennis Carroll <dcarroll@usaid.gov>

Hi, Jonna,

Can you and the PREDICT team help with this request from CDC? If you have tools/resources that you think would be appropriate to share, could you send them to me? I'd like to compile everything from the EPT-2 partners and send it as one e-mail.

Thanks,

Ricardo

– –

Ricardo Echalar, MPH
Senior Public Health Advisor
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---------- Forwarded message ----------
From: **Barton Behravesh, Casey (CDC/OID/NCEZID)** <dlx9@cdc.gov>
Date: Wed, Jun 21, 2017 at 2:38 PM
Subject: REQUEST FOR MATERIALS: GHSA Toolkit
To: "Ricardo Echalar (rechalar@usaid.gov)" <rechalar@usaid.gov>
Cc: "Goryoka, Grace (CDC/OID/NCEZID)" <lie0@cdc.gov>, "One Health (CDC)" <onchcalth@cdc.gov>, Sarah Paige <spaigc@usaid.gov>

Dear Ricardo,

CDC is creating a repository of tools and resources across GHSA countries and Action Packages. This repository will be accessible to CDC field missions, CDC headquarters, and in-country partners to support program design, work plan development, monitoring and evaluation, and technical assistance. Please find the one-pager attached for more information.

Support for this repository has broadened following the call for tools and resources at the *Multicountry/Multisector Partner* plenary session at a recent CDC global health meeting (the DGHP Annual Meeting). We are reaching out to partners and countries for the tools and resources they use to implement GHSA activities. I wanted to specifically reach out to our One Health partners for information.

Understanding the importance of your subject matter expertise, we want to ensure we include tools and resources that CDC perceives as critical to GHSA. We invite you to share policies, standard operating procedures, M&E frameworks, and other tools and resources that you perceive as good examples for countries and partners to reference.

If there are materials you would like us to share with this repository (i.e. overview of the NOHPs), please send it to onehealth@cdc.gov no later than June 28th. We will turn in all materials we received to the repository. Also, as future materials become available, we can add to the repository to share One Health information widely. I thought it would be useful to share the NOHP summary overview as a resource in this tool kit as a way to provide CDC country staff with key information on the NOHPs. You are welcome to share additional materials that you think would be useful. Eventually, we can add the One Health interagency talking points.

Many thanks,

Casey

Casey Barton Behravesh MS, DVM, DrPH, DACVPM

Captain, U.S. Public Health Service

Director, One Health Office

National Center for Emerging and Zoonotic Infectious Diseases

Centers for Disease Control and Prevention

e-mail: CBartonBehravesh@cdc.gov

www.cdc.gov/onehealth

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Section 5.2.9. Small Carnivore Sampling Methods

Prepared by
Christine Fiorello, University of California, Davis
Marcela Uhart, University of California, Davis
and the PREDICT One Health Consortium

Objective: To safely collect biological samples from live and dead small carnivores.

This document was made possible by the generous support of the American people through the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT program. It was drafted to support activities conducted under PREDICT and is intended for an audience of qualified professionals trained in standard, associated best practices. This guide is not intended for use by untrained individuals.

The authors assert that animal capture and sampling should always occur in compliance with all applicable laws and regulations and should only be undertaken after securing all necessary permits and approvals, including ethical approvals.

For more information about the contents of this guide, please contact predict@ucdavis.edu.

Suggested Citation Form: PREDICT One Health Consortium 2016. PREDICT Operating Procedures: Small Carnivore Sampling Methods.

Table of Contents: Small Carnivores

Section 5.2.9a. Confirmation of Knowledge

When you are familiar with the information in this Guide, take the PREDICT quiz ***Section 8.4.8. Small Carnivore Sampling***.

Section 5.2.9b. Brief Overview of PPE

Minimum PPE Required for Handling Small Carnivores

The minimum PPE for sampling small carnivores includes:

- Designated clothing
- Nitrile gloves
- Protective glasses
- N95 facemask for self-protection and to avoid contaminating samples

(See the ***Biosafety and PPE Guide (Section 4.)*** for detailed instructions regarding PPE Use)

Note*: In order to protect both human handlers and sampled small carnivores, all personnel handling small carnivores should be vaccinated against rabies beforehand as described in the following Section.*

Section 5.2.9c. Special Considerations for Handling Small Carnivores

This section supplements ***Section 5.2.5. Safe Animal Capture and Sampling***, with which anyone handling animals is expected to already be thoroughly familiar. Note also that sampling from dead animals, whether destined for bushmeat or not, is also covered in ***Section 5.2.12. Bushmeat Sampling Methods***. However, for completeness, much of that protocol is repeated here.

Handling small carnivores involves a number of special considerations:

1. Zoonotic diseases
2. Staff vaccinations and medical concerns
3. Other hazards (bites and scratches)
4. Capture and handling

1. Zoonotic Diseases

There are numerous zoonotic diseases that may be transmitted from small carnivores to humans. Here we highlight the most important diseases that are a risk to human handlers. Research teams should be familiar with additional zoonoses that may be present in their target and bycatch species and geographic areas. It is recommended that immunocompromised people not work directly with live or dead animals.

Rabies is endemic in many carnivore populations, and may show up sporadically in any carnivore. Therefore, anyone who expects to handle live or dead carnivores should be appropriately vaccinated. Clinical signs of rabies are quite variable and can include any neurologic abnormality; this includes depressed mentation, which is a general sign displayed by nearly all sick or injured animals. When handling a carnivore, the potential for rabies should be assumed and appropriate precautions taken. Most zoonotic exposure is via bite wounds from live carnivores. However, exposure to infected bodily fluids (particularly saliva, blood and cerebrospinal fluid) must be considered when collecting samples from dead carnivores. Rabies is not transmitted by casual contact. Many other viruses can also be zoonotic.

Leptospirosis is a bacterial disease that affects a wide variety of mammals. It causes septicemia, kidney, liver, pulmonary, and/or reproductive dysfunction and is spread by contaminated water and urine. Gloves and good hygienic practices (i.e., thorough hand-washing) should provide protection.

Salmonella spp. are bacteria that can cause severe disease in humans and carnivores, but can also be carried by these species asymptomatically. *Salmonella* can cause septicemia as well as diarrhea, and all carnivores are potential carriers. Special care should be taken when working with animals, live or dead, that have evidence of diarrhea. *Salmonella* spp. are most likely to be shed in the feces, but fur, traps, and vegetation where animals are held can all become contaminated. For that reason, gloves should be worn while handling animals and while cleaning/handling traps and cages in which animals have been captured or housed.

Mycobacteria bovis, the cause of bovine tuberculosis, is a bacterium sometimes found in carnivores that prey on species in which *M. bovis* is endemic. The most likely exposure for staff would be during necropsy, so gloves and mask should be worn during necropsies of carnivores in endemic areas (i.e., East Africa). This disease is of special concern for those who are immunosuppressed, but it can cause disease in immunocompetent individuals as well.

***Echinococcus* spp.,** a cestode parasite of canids and their mammalian prey, causes disease in humans when the eggs are ingested. Infective eggs are shed in the feces by the carnivore definitive hosts, which includes domestic dogs as well as wolves, foxes, jackals, lions, and sometimes other canids and felids. The disease is caused by large cysts formed by the larvae; cysts are most commonly found in the liver and lungs. For prevention, gloves should be worn and hands should be thoroughly washed after handling carnivores or items that could be contaminated with carnivore feces.

Parasites, both external and internal, may be transmitted from carnivores to humans. Internal parasites are most commonly transmitted via the feces, so precautions taken for salmonellosis should also protect against these organisms. External parasites, such as *Sarcoptes* mites (the cause of mange) and fleas, causing dermatitis, may also transmit pathogens (e.g., fleas may carry *Yersinia pestis*, the causative agent of plague). Wearing gloves, long sleeves and pants while handling carnivores can help prevent transmission of parasites. When performing necropsies on heavily infested carcasses, the carcass may be dusted with an acaricide prior to handling.

A variety of other pathogens may be transmitted from wild carnivores to humans, and risk of exposure varies by geography and other factors. Wearing gloves, good hand washing protocols, and common sense hygienic practices will protect against transmission of most pathogens. Personnel working with animals should always wash their hands thoroughly before eating, drinking, using tobacco products, or any other activity that involves touching the face or mucus membranes.

2. Staff vaccinations and medical concerns

All those working with carnivores should be vaccinated against rabies, ensure that they have a protective titer, and be aware of appropriate post exposure prophylaxis in the case of bites.

3. Bites and scratches

With few exceptions, carnivores are predators and have formidable weapons. Small carnivores can inflict powerful bites that cause massive tissue damage and inoculate bacteria deep into tissue. Most carnivores have claws on all four feet and felids, especially, can do severe damage with their claws. Small carnivores such as civets, otters and mongooses should not be underestimated; they are remarkably agile and cannot be safely sampled without chemical restraint. No one should attempt to work with larger carnivores without proper training and a healthy respect for the risk in working with these species.

4. Capture and handling

Most carnivores are captured with traps, such as box traps, foothold traps, or snares. All traps, regardless of padding or safety features, pose some degree of hazard to the animal. Carnivores are capable of severe self-injury in any type of trap; they can break canine teeth or tear off claws in box traps, or chew off limbs caught in foothold traps. Snares set for large animals have obvious risks for smaller carnivores. For these reasons, traps should be prepared carefully, in good working order, and checked frequently, at least every 12 hours. Veterinary staff should be prepared to deal with a variety of trap injuries, including lacerations, broken teeth, and crush injuries.

Many carnivores captured in footholds or snares can be dangerous to personnel. Trapped animals should be approached carefully. Most species will need to be chemically immobilized by blowdart or pole syringe, but some smaller animals (such as some jackal species) can be handled with manual restraint and tools such as gloves and nets. Animals in box traps can be hand-injected or pole-syringed. Ketamine hydrochloride combined with a tranquilizer (such as a benzodiazepine or an alpha-2 agonist), or Tiletamine-Zolazepam (Telazol, Zoletil) are the mainstay of carnivore immobilization protocols, but many protocols have been used and protocol success varies by species. Appropriate protocols for individual species can be found in various publications such as Kreeger, Fowler, Fowler and Miller, Nielsen, and West, Heard, and Caulkett (See References Section). Chemically immobilized animals should be housed in a trap or cage until they are fully recovered from anesthesia to prevent injury or death from drowning, falls, predation, or intraspecific aggression. While many protocols include one or more reversible drugs, neither ketamine nor the tiletamine component of Telazol are reversible, so protocols containing either of these drugs are **not** fully reversible. Therefore, giving a reversal agent is not a guarantee of an immediately awake and aware animal. Animals should be observed carefully to ensure that they are fully recovered before they are released, keeping in mind that a reactive animal is not necessarily a completely awake animal. Animals are typically ready for release when they can hold their head steady and follow movements with their eyes.

Care should be taken to decrease the stress experienced by animals prior to and during immobilization. This includes keeping your distance from the captured animal, speaking in soft voices and minimizing noise, and covering box traps with a towel or tarp to decrease visual stimuli. Similarly, anesthetized animals should have their eyes covered with a towel or cloth. It is especially critical that immediately after initial drug administration, the animal is free (to the extent possible) of visual and auditory stimuli; the quality of the induction often impacts the whole anesthetic event. During recovery, keep the caged animal within view, but at a great enough distance that the animal does not feel threatened by your presence. As always, noise should be kept to a minimum.

Anesthetized animals should be monitored regularly during recovery until they can no longer be safely handled, at which point they should be confined in a trap or cage. Essential monitoring includes measuring and recording heart rate, respiratory rate, body temperature, mucous membrane color, and pulse quality every 5-10 minutes throughout the procedure. The eyes should be lubricated with a bland ophthalmic ointment and protected from debris. Animals should be kept out of direct sunlight and overheated animals (>105°F/40.6°C) should be cooled by placing rubbing alcohol on their paws, administering cool subcutaneous fluids, or wetting their fur. Cold animals (<100°F/37.8°C) should be kept warm using tarps, blankets, or warm SQ fluids. Emergency drugs, appropriate-sized endotracheal tubes, and a mechanism for providing positive pressure ventilation (i.e., Ambu bag) should be available whenever animals are immobilized.

Section 5.2.9d. Small Carnivore Data Collection

Please refer to the three required data collection templates for data to collect. These include:

1. P2 Animal Data Collection Form
2. P2 Site Characterization Data Collection Form
3. P2 Specimen Data Collection Form

For more information on downloading templates from EIDITH see ***Section 5.2.3. General Data Collection Templates and Applications***.

In some cases time constraints, anesthetic risk, inability to prolong immobilization, or other factors may necessitate prioritizing biological sample collection at the expense of collecting any physical measurements. At a minimum:

1. Measure and record the animal's mass (kg) initially as this can be important for proper drug dosing or emergency interventions.
2. Conduct a cursory physical exam before sampling in order to note any wounds or major abnormalities and to protect the health of both handler and animal.
3. Ensure the animal's parameters (heart and respiration rates, body temperature, etc.) are adequate for continuation of procedures. If they are not, attempts to correct them should be made and reversal of anesthesia considered if the animal's life is at risk.

<u>Additional (Optimal) Data to Collect from Small Carnivores</u>

The P2 data templates mentioned above are required to be filled in. Additional data and biometric measurements may be collected at the discretion of the sampling party.

Ideally, the following additional data should be collected from any small carnivores that are processed for PREDICT:

1. Body mass (weight)

Body weight: Although in an ideal world the body weight of an animal would be measured prior to drug dose calculation, because of the dangerous nature of carnivores this is rarely possible in field conditions. Weight ranges of the target (and likely non-target) species should be known before capture is attempted, and the veterinarian or biologist should have experience estimating weights. Animals should be weighed (g or kg) in bags, slings, or a suitable container using a calibrated hanging spring. (Note: If an animal exceeds the limit of spring scales, two or more scales can be linked (one hanging from the other) to distribute the weight. The total weight is the measure of both scales added together). Scales should be zeroed (checked to make sure they measure '0.0' units when empty). If scales are not available or accurate weights cannot be measured for any other reason, a weight should still be estimated but the recording sheet <u>MUST note that it is an estimated and not a measured weight</u>.

2. Age class (see below)

Age class: Usually the exact age will not be known. Individuals should be placed into one of the following age classes:

Age Class	Description
Juvenile	Dependent young, likely unweaned
Subadult	Animal is fully independent, appears to be sexually mature, but not fully physically mature (e.g., less than full adult size).
Adult	Animal has secondary sexual characteristics, adult size, sexually mature.
Old Adult	Adult showing signs of age degeneration (i.e. tooth wear)

3. Species Identification and Sex Determination (and reproductive status if adult female)

Species identification and sex determination: Based on morphology and unique characteristics, identify animal to genus and species (where possible) and sex. If dependent offspring are captured along with their mothers, they should be kept confined while their mother is anesthetized to prevent them from wandering off. Reintroduce them only when the mother is fully recovered.

4. Whole body photograph(s)

Photographs: At a minimum, the following digital photographs should be taken of each individual:

a. Right and left lateral views while animal is recumbent.
b. Full anterior facial view.
c. Full lateral facial/head view.
d. Views of full upper and lower dentition (which can help determine/verify age and sex).
e. Views of any lesions (e.g., cuts, scratches), physical abnormalities (e.g., missing toes), or individually identifying marks or characteristics (e.g., healed scars, abnormal coloration, torn ears, etc.)

5. Morphometric measurements

Body measurements: Time required for collecting the biometrics (in cm/mm) should be recorded with the <u>minimum standard mammal measurements (all linear) as follows:</u>

a. Head and body length (measured dorsally and linearly from tip of nose to base of tail when head is stretched and aligned with back).
b. Tail length (from base to tip).
c. Hind foot length (heel to tip of longest toe- exclude nail and note which toe).
d. Tibia length ('knee to ankle').
e. Hind foot (tarsal) length
f. Ear length- base of the notch below the ear opening (lower rim of external auditory canal = meatus) to the most distant point of the margin of the pinna.

Additional Optional Measurements
Head length, trunk height, hip breadth, hand length and breadth, foot breadth, limb segments (thigh, lower leg, upper arm, forearm).

Chest circumference, abdominal circumference, and cranial circumference (at or above brow).

Section 5.2.9e. Small Carnivore Sampling

Capturing, trapping, darting, and immobilizing small carnivores should only be performed by experienced and skilled staff.

PREDICT personnel are expected to have detailed capture/immobilization protocols (and recording sheets, monitoring sheets, etc.) for any target small carnivore species. This sampling guide assumes a starting point of either a safely immobilized or an already dead small carnivore.

In addition to the standard PREDICT sampling and analyses, PREDICT partners are encouraged to collect additional samples and pursue routine diagnostics (e.g., blood counts and chemistries, urinalysis, etc.) where resources allow. Opportunities to collect biological samples and related health data from wild animals are uncommon and maximizing these opportunities can further advance wildlife health knowledge.

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two oral swabs** - one in 500 µL VTM and one in 500 µL Trizol
2. **Two fecal samples** - one with max of 500 µL/0.5cc feces in 500 µL VTM and one with max of 500 µL/0.5cc feces in 1 mL Trizol
   Or
   Two rectal swabs - one in 500 µL VTM and one in 500 µL Trizol
3. **Two whole blood samples** - one with max of 500 µL of whole blood in 500 µL VTM and one with max of 500 µL of whole blood in 500 µL Trizol
4. **Two serum samples** - 2 x 0.5 ml aliquots frozen without media
5. **Two urogenital swabs/urine samples** – one in 500 µL VTM and one in 500 µL Trizol

***Note**: If animals are too small to collect two blood tubes (for whole blood and serum), collect serum and save remaining clot in 500 µL VTM after serum separation.*

Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

If there is no **short-term** access (i.e., within 24 hours) to cold chain such as in an emergency situation then samples can be collected in 500 µL of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

1. 1 day at 37 °C (i.e., ambient temp)
2. 1 week in the refrigerator
3. Within one week freeze at -80 °C for storage until analysis

Sample Labeling

Tubes must be labeled with a unique specimen ID per Animal/specimen labeling guide. Please see ***Section 5.2.3. General Data Collection Templates and Applications*** for details about assigning Animal IDs.

Sample Collection from Live Small Carnivores

In most cases, live small carnivores should be chemically restrained for handling. At least two, and preferably three people are required for these manipulations: one person to monitor the animal, one to take samples, and a third to manage the tubes and record data.

1. **Two oral swabs in VTM and Trizol** (if only one is collected, place sample in VTM): Using sterile, polyester-tipped swabs with a plastic shaft, rub the swab tip gently but thoroughly against the back of the animal's throat, saturating the swab with saliva.

   Place 1 swab in a cryovial filled with 500 µl of VTM and use a flame-sterilized scissors to cut the shaft of the swab above the tip. Place the other swab into 500 µL of Trizol in another cryovial and cut shaft as above. [Note: If the plastic shaft can be snapped, then scissors are not necessary and the risk of cross-contamination is reduced. To snap the swab, lift the swab a little above the bottom of the vial then snap it. This will ensure the swab will not block the cap].

   Mix each tube well. Store both cryovials in a liquid nitrogen dry shipper or dewar and transfer to -80˚C freezer when possible.

2. **Fecal samples**
   500 µL or pea-sized piece of feces (200 mg) in VTM and Trizol: Collect either excreted feces, or <u>if animal is large enough (> 1 kg)</u> use a gloved, lubricated (saline or medical lubricant) finger to collect feces directly from rectum. Place two ~200 mg (pea size) samples of fresh feces into 2 vials, one containing 1 mL Trizol (= maximum final ratio of 1:2) and one containing 500 µL VTM (= maximum final ratio of 1:1). Homogenize by shaking. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80˚C freezer when possible.

   If feces are not available, collect **2 rectal swabs, one in VTM and one in Trizol:** Gently insert one sterile swab tip at a time into the animal's rectum. Rectal swabs can be moistened with sterile saline prior to animal sampling. [Note: DO NOT USE TRIZOL AS A LUBRICANT – IT IS HIGHLY IRRITATING TO TISSUE. Also, DO NOT USE VTM AS A LUBRICANT.] Place one swab in a cryovial filled with 500 µL of VTM using a flame-sterilized scissors to cut the shaft of the swab above the tip. Place the other swab into a tube with 500 µL of Trizol. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80˚C freezer when possible.

3. Whole blood and serum samples

Precautions

- **At least one person present should have previous experience in small carnivore venipuncture to avoid injury to the animal.**
- Animals should be immobilized using either injectable or gas anesthesia according to appropriate guidelines. On occasion some species may be manually restrained for venipuncture but extra care must be taken to avoid injuries to personnel and animals.
- The person restraining the animal is responsible for monitoring respiration and other vital signs and communicating the status of the animal appropriately.
- No more than 1 ml of blood per 100 g (= 10 ml/kg or 1%) of body weight should be collected at any one time; it is best to limit collection to 0.6 ml blood per 100g.
- Blood should always be considered highly infectious and hazardous.

Collection Procedure

1. Select appropriate venipuncture site:

Animal family	Venipuncture site
Felids	
	Medial saphenous vein: With compression of the inner thigh, this vein can be prominent and superficial, but often collapses during collection. Use of a butterfly needle and extension set may help avoid this problem, as well as using a smaller syringe and pulling back slowly on the plunger.
	Cephalic vein: In larger species the cephalic vein might be large enough for safe blood collection.
	Jugular vein: This may be the only option in very small animals and must be accessed carefully.
	Lateral tail vein: This may be accessed in larger felids; the same comments as for the medial saphenous vein apply.
Canids and Hyenids	
	Lateral saphenous vein
	Jugular vein
	Cephalic vein
	Femoral vein: It is easy to hit the femoral artery instead; if this happens,

be sure to apply firm, direct pressure for several minutes to effect hemostasis
Medial saphenous vein: May be accessible in some species

Mustelids

Jugular vein: Due to their short muscular necks, this can be a difficult vein to access. Placing pressure in the thoracic inlet bilaterally often helps to occlude this vein and help it pop up.
Femoral/saphenous vein: These tend to be short and difficult to hit, and the femoral artery can be accidentally hit as in canids and hyenids.

Viverrids and Herpestids

Jugular vein: Best option for adequate samples
Cephalic: Small volumes
Tail (ventral midline): Small volumes

Ursids

Cephalic vein
Saphenous vein
Jugular vein

Note: *When using an alpha-2 agonist in a chemical immobilization protocol, the peripheral veins often collapse due to vasoconstriction. Using the jugular vein will often be necessary for venipuncture.*

2. Select appropriate sized needle and syringe (or vacutainer) for the size of the animal.
3. Disinfect the site with iodine solution or alcohol.
4. Collect sample.
5. **Do not recap needle**.
6. Apply pressure to site of bleeding using a cotton ball or gauze pad until bleeding ceases (approximately 1 minute).
7. Process blood (see below).
8. Properly dispose of sharps and other biohazard materials immediately upon transfer of sample to collection vials.

Blood Processing

a. **Whole blood in EDTA:** Collect 1 tube of whole blood in EDTA (lavender top vacutainer). Add up to 500 µL of whole blood into 2 vials, one containing 500 µL Trizol and one containing 500 µL VTM (= maximum final ratio of 1:1) and mix each vial well. Place vials into liquid nitrogen in dry shipper or dewar and transfer to -80˚C freezer when possible.
b. **Aliquot serum into cryovials**: Collect blood into a serum tube (red top or tiger top, if >1 mL blood is collected, or into 1.5 mL conical Eppendorf tubes). Place labeled blood tubes

in a rack on ice (optimally) for up to 2 hours prior to centrifuging. Centrifuge the blood samples. If a centrifuge is not available, red top tubes with blood can be left standing on ice overnight to allow serum to separate. Use a pipette to draw off serum, aliquot into 0.5 mL volumes per cryovial, and store cryovials in a dry shipper or dewar. As soon as possible, remove samples and place in cryoboxes and store in an -80 °C freezer.

4. Urine

Collect 2 urogenital swabs and place one in 500 µL of VTM and one in 500 µL of Trizol. Swabs can be moistened with sterile saline prior to animal sampling. Alternatively, if the animal urinates, urine may be collected by free catch from the urethra. Aliquot up to 500 µL of urine using a pipettor into one cryotube and add 500 µL of VTM. Add another sample of up to 500 µL of urine into another cryotube with 500 µL of Trizol. Mix cryotubes well.

Sample Collection from Dead or Euthanized Small Carnivores

If carcasses are not whole, then the ***Bushmeat Sampling Methods (Section 5.2.12.)*** may be more applicable. If bodies are relatively whole and fairly fresh, then sample as described above. If an animal must be euthanized due to humane or veterinary care reasons, see ***American Veterinary Medical Association guidelines (Section 8.5.2.)***.

As discussed throughout this protocol, all wildlife should be considered potentially infectious for a wide variety of dangerous pathogens and dead animals in particular should be sampled only following all safety measures including proper PPE use, proper work station decontamination, and proper carcass disposal as outlined here and in other PREDICT documents.

Though not required for PREDICT sampling, thorough necropsy procedures can be very beneficial and might pertain to some animals (e.g., valuable or known individuals, suspicious deaths, etc.). Necropsy protocols are addressed in separate documents. Time and skill permitting, when full necropsies are performed, following any Association of Zoos and Aquariums/AZA (or similar) necropsy protocol is recommended and most can be adjusted for application to other species. (*Note that properly following extensive necropsy procedures and collecting and measuring all samples can require 4-6 hours for a single animal.*)

Post-Mortem Blood Collection

From recently dead animals, it may be possible to collect whole blood (often clotted) from the right side of the heart where the largest volume of blood is available. Collect all available blood into an appropriate size container (typically one or more blood tubes). Allow the tubes to sit undisturbed for at least 30 minutes, and then centrifuge at high speed (2000 x G for 20 minutes). Transfer the serum (clear, yellow or red-tinged fluid at the top), preferably via pipetting, to appropriately labeled cryovials. Transfer the remaining blood clots to separate cryovials. Refrigerate or freeze both the serum and blood clots.

If a centrifuge is not available, allow the clots and cells to settle as much as possible, and then collect the serum and clots as described above. If the animal's death is recent enough that the blood has not yet clotted and a centrifuge is not available, invert the blood tubes after the blood has been collected to allow the clot to form on the rubber stopper. After the blood has clotted, turn the tube right side up and carefully remove the stopper with the adhered clot, thereby leaving a clean serum sample in the tube.

At a minimum, as many of the following blood samples as possible should be collected:

- 2 samples of 500 µL (**whole blood**) placed in 2 vials, one containing 500 µL **Trizol** and one containing 500 µL **VTM** (= maximum final ratio of 1:1). Mix each vial well. **If only one sample can be collected, then place it into VTM.**
- 2 or more aliquots (0.5 ml) of **separated serum**, frozen

Tissue Collection

Collect **three**, adjacent, approximately 200mg (pea-sized) samples of the following tissues:

- Adrenal
- Colon
- Heart
- Liver
- Lymph node
- Ovary
- Testes
- Cecum
- Duodenum
- Kidney
- Lung
- Spleen
- Pancreas
- Other, if required*

One specimen should be frozen in 500 µL VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment). **If only one sample can be collected, then place it into VTM.**

*It will usually require experience to identify abnormal tissues, but potentially recognizable gross lesions include masses, discolored areas, ulcerations, etc. Samples for histopathology (i.e., in formalin) should be collected at the abnormal margins to include both normal and abnormal sections in the same piece of tissue. Collection of any obvious internal parasites in ethanol is also recommended.

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Section 5.2.5. Safe Animal Capture and Sampling

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Objective: To provide principles and general considerations for the safe capture of wild animals and safety of personnel during these captures.

This document was made possible by the generous support of the American people through the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT program. It was drafted to support activities conducted under PREDICT and is intended for an audience of qualified professionals trained in standard, associated best practices. This guide is not intended for use by untrained individuals.

The contents of this document are the responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government. USAID, PREDICT, and the authors of this guide bear no responsibility for the actions of non-PREDICT-affiliated individuals implementing the material herein.

The authors assert that animal capture and sampling should always occur in compliance with all applicable laws and regulations and should only be undertaken after securing all necessary permits and approvals, including ethical approvals.

For more information about the contents of this guide, please contact predict@ucdavis.edu.

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Table of Contents: Safe Animal Capture

Section 5.2.5a. Learning Objectives and Confirmation of Knowledge

If you understand the material in this Guide, you should be able to:

- Explain the PREDICT ***Bushmeat Sampling Methods (Section 5.2.12.)***.
- Safely participate in animal capture activities.
- Identify potential personnel risks of injuries and risks of exposure to zoonotic pathogens associated with the capture of wild animals.
- Develop a personnel safety plan for animal capture activities.
- Identify potential risks of injury or harm to animals that may be captured and identify ways to minimize those risks.
- Identify potential risks for captured wild animals of exposure to human pathogens during restraint and sampling, and develop ways to minimize those risks.
- Explain the special hazards associated with the use of anesthetic drugs and other chemicals used in animal capture.
- Explain important precautions to protect animals when the animals are anesthetized or physically restrained.

Confirm you understand the material of this Guide

When you are familiar with the information in this Guide, take the PREDICT quiz ***Safe Animal Capture and Sampling (Section 8.4.4.)***.

Section 5.2.5b. Principles & Guidelines for Personnel Safety & Health During Wild Animal Capture

General Principles on Personal Safety

Capture, handling, and anesthesia of wildlife during field projects are often carried out in remote areas away from medical assistance. Therefore, every possible effort must be made to prevent injuries to personnel. The following precautions should be considered for fieldwork that involves handling animals for diagnostic sample collection:

Field teams, particularly animal capture teams, must be prepared to deal with potentially hazardous situations and have contingency plans in place to respond to accidents, injuries or other unexpected circumstances.

Investigators implement measures, in accordance with protocols or established guidelines, to protect their staff, co-workers and themselves against possible injury or exposure to potentially dangerous procedures, drugs, chemicals, animals, or animal fluids and waste.

Investigators must clearly identify and discuss with project personnel the hazards to human health and safety and the appropriate safety precautions to be taken when working with wild animals.

Investigators should ensure that all project personnel are properly trained, have written procedures, and have the appropriate protective clothing and personal protective equipment (PPE) for their safety.

Investigators should familiarize themselves with known biohazards specific to the species under study and with the procedures to avoid exposure to these agents.

Personnel should work in teams of at least two people in the field, especially when involved in physical or chemical restraint and handling of animals or other high-risk situations.

If an animal becomes difficult to handle safely, the handler should release the animal if it is safe to do so. Additional restraint, chemical or physical, may be needed to adequately control the animal safely (see section below).

Keep an open route of escape when working with animals.

Personnel may need to wear protective clothing including protective footwear with non-slip soles, sturdy clothing (e.g., long-sleeve shirts, long trousers, plastic aprons, etc.), gloves, and face masks. The appropriate protective clothing depends on site and species-specific field conditions.

Field workers should be trained in the tasks and safety procedures relevant to the animal capture and handling activities, including how to avoid injury from equipment or animals and how to avoid exposure to potential pathogens. They should also be trained to avoid transmission of human pathogens to captured animals.

Special considerations:
Individuals with known allergies associated with animals, with immune deficiency diseases, or on immunosuppressant therapy, should not engage in studies involving the handling of wild animals.

Certain animals, including but not limited to bats, dogs, and non-human primates, are known to harbor disease agents considered to be deadly to humans such as rabies. Any capture and manipulation of these animals warrant that the handlers wear double gloves, catching gloves (if necessary), and immediately report any bites or exchange of blood/fluids to supervisors, field coordinator**,** or medical professionals if present in the area. Preventative vaccination is recommended.

General Guidelines on Safe Handling of Animals

Supervising veterinarians and other trained PREDICT staff will handle animals as part of surveillance activities and sampling fieldwork. Staff must be trained on the potential hazards and safe handling techniques for the specific types of animals they are likely to handle. Animal hazards may include injuries due to sudden animal movements, bites and scratches, and exposure to zoonotic pathogens. The following precautions should be considered for the safe handling of animals:

Handlers should have a basic understanding of the animal's typical behavior.

All animal handlers should be trained in basic animal handling techniques and those techniques should be used consistently. Improvements to techniques should be tested and implemented when available.

Generally, slow and deliberate movements should be used around animals.

Animal behavior can be unpredictable. Therefore, personnel should remain constantly alert when handling wild animals. Personnel should watch for warning signs of animal aggressiveness and fear. These signs vary with animal species and may include vocalizations, raised fur, flattened ears, twitching tails, or bared teeth. An animal that feels threatened or cornered may be more aggressive than under normal circumstances.

Extra caution should be used when handling animals that are sick, hurt, or are new mothers or highly territorial species.

If capture and sampling procedures may cause pain, animals should be handled safely and humanely under the supervision of a veterinarian trained in wild animal chemical immobilization and animal restraint devices.

Workers should use extreme caution when giving injections and handling sharps around animals; sudden animal movements could cause a needle stick injury to the personnel or injury to the animal's vital organs.

Investigators should be aware of the potential for human pathogen transmission to wildlife and ensure adequate use of protective equipment to avoid exposing animals. In addition, sick workers should not be allowed to participate in animal handling.

Safe Operation of Equipment

All personnel involved in wildlife capture should have current training in the use of pertinent equipment, including but not limited to different kind of traps, nets, and snare poles (rabies-poles used for capture and restraint. Specific on appropriate capture and handling of PREDICT target species are described in the taxa-specific PREDICT protocols. Overall field team knowledge on the correct use of equipment will help to minimize injury due to accident or the misuse of equipment during animal capture and handling. Use of drug delivery equipment, such as dart rifles, dart pistols and darts, jab-sticks (pole-syringes) must be done under the supervision of a veterinarian trained in wild animal physical and chemical restraint.

Safe Use of Anesthetic Drugs and Other Chemicals

Capture of free-ranging wildlife may place personnel at risk of injury and anesthesia or other forms of chemical immobilization may be necessary. All use of drugs or chemicals must be done other the supervision of a veterinarian trained in wild animal physical and chemical restraint. Injury can occur, not only from animal attacks, but also capture equipment, or exposure to potent drugs. Every possible effort must be made to minimize the probability of human injury when undertaking chemical restraint and/or anesthesia of wildlife. The following precautions should be followed when using anesthetic drugs or other chemicals:

The risks involved in using drugs for the capture and immobilization of wildlife must be identified and communicated to all project personnel.

At least two people on the team should be trained in first aid and cardio-pulmonary resuscitation (CPR) (one of the two should be a person NOT in charge of handling anesthetics). First aid or CPR may be required in an accidental drug exposure emergency. A well-stocked first aid kit customized for each project should be kept within easy reach during fieldwork activities.

An evacuation plan for an anesthetic drug related accident should be developed and communicated to all field personnel. Local medical authorities should be informed of the potential hazards of the field work and an evacuation plan to medical facilities should be discussed prior to beginning fieldwork.

All drugs and chemicals used in field research should be handled in such a way as to prevent human exposure. Researchers or personnel authorized to use immobilization drugs should protect themselves against eye, respiratory and cutaneous exposure to drugs and chemicals and accidental injection. The use of gloves, long-sleeve clothing, and protective goggles and/or face-shields may be indicated in some cases.

Those utilizing immobilization drugs for restraint of wild animals should have the appropriate training and information available to aid in their medical care should accidental contamination occur. It is advisable to always have drug manufacturer information for all medications in use as some human emergency facilities will not be familiar with drugs used for the immobilization of wildlife particularly in countries where such drugs are not available commercially.

It is recommended to work in pairs when utilizing highly potent drugs, so that there is constant monitoring of the person handling anesthetics. When anesthesia drugs being used have an appropriate reversal agent, both people should carry a full dose of reversal drugs (for human treatment) at all times. There should be adequate quantities (for human treatment) of reversal drugs on hand in the field if these exist. Potent anesthetic drugs should be handled only by trained veterinarians.

When darts are used to restrain animals, every reasonable attempt should be made to recover all darts that miss the target animal as they contain chemicals that could pose a public or animal health risk. As with syringes used to draw up medications, darts should be placed in a special container to avoid accidental exposure to personnel.

National and local regulations with regard to drugs, specialized equipment (rifles, pistols), and liability issues concerning medical treatment of humans and/or human well-being should be clearly understood and followed before fieldwork begins.

Biohazards and Zoonotic Diseases

Investigators and field workers are at risk of exposure to zoonotic diseases (diseases transmitted from animals to humans). Pathogen transmission may occur through direct contact with contaminated dirty hands and equipment, bites and other direct exposure to animal fluids (blood, urine, saliva) or inhalation of contaminated dusts. Investigators and supervisors should familiarize themselves with known biohazards specific to the species under study and with the procedures to avoid exposure to these agents as detailed in the taxa-specific protocols.

Prior to fieldwork, the Country Coordinator or field supervisors should provide training and information regarding all potentially hazardous biological or zoonotic agents that may be encountered in the field situation or that are relevant to the species under study.

Personnel must wear the specified PPE, as indicated for each taxonomic group. Additional PPE could be warranted given certain field situations, and or as determined by the PREDICT field supervisor. PPE is required to prevent bites or scratches. Protective eyewear and respirators is also needed to prevent exposure to pathogens transmitted by splashing of body fluids or secretions, or inhalation of contaminated aerosols.

The Country Coordinator or field supervisor must ensure that safety procedures are established for the conduct of postmortem examination in the field and that appropriate protective equipment (e.g. aprons, gloves, face-masks, eye protection, etc.) is available and used correctly. The Country Coordinator is responsible for ensuring that all personnel be trained in the postmortem techniques appropriate for the species.

Field workers should wash hands or use disinfecting hand wipes frequently before and especially after animal capture and field activities. Frequent handwashing is the best defense against diseases transmitted through contact with contaminated animal saliva, other body fluids and wastes.

If injured by an animal or potentially exposed to a diseased animal, workers should immediately report to their supervisor and/or coordinator and seek the appropriate medical attention and follow-up. See ***Section 3. Emergency Preparedness*** for detailed instructions and accident reporting forms.

Field personnel should also take precautions (i.e. long sleeve shirts, insect spray, etc.) to avoid exposure to external animal parasites such as ticks, fleas, as well as to animal feces that may contain internal animal parasites (ova or larvae) infective to humans.

If personnel become sick or show unusual symptoms they should immediately report this to the supervisor and should contact medical authorities knowledgeable about the diseases and parasites in the region. Field staff should discuss animal and field exposures and potential zoonotic hazards with their medical care staff.

Immunizations and Pre-Exposure Screenings

The Country Coordinator or field supervisor should ensure that personnel have consulted with a human health worker with regard to the immunizations required prior to participating in fieldwork that involves handling animals. See ***Emergency Preparedness (Section 3.)*** for details and immunization forms.

Vaccines and immunizations will vary depending on the geographical area, animal species to be handled, and personal medical history. Only a human health professional can provide vaccination and immunizations to the staff.

Due to the significant risks of working with wild mammals (bats, rodents, etc.), field personnel should be required to receive pre-exposure rabies vaccination (before starting any field project) for themselves. Tetanus immunization should also be required for all staff that will have any contact with wild animals.

Pre-exposure screening for tuberculosis is required for personnel that will be handling non-human primates. Tuberculosis screening and interpretation of results should only be conducted by a human health professional.

Training Records

A record must be kept of all training given to PREDICT personnel and reported into the EIDITH system for tracking training activities. For each training activity (on-the-job, self-study, small group training, workshop) the following information should be reported into the EIDITH system for tracking training activities (see ***Section 8.3.1. Training Management Forms)*** for training forms and directions on how to enter data into EIDITH):

- Date of training
- Type of training (workshop, on-the-job, simulation, field or lab training)
- Location of training (town and country)
- Topics covered
- Instructor(s)
- Trainees' names, gender, phone, email, title, organization, and sector

Country Coordinators and field supervisors should maintain a record (on the PREDICT report form) of any injuries or illnesses incurred while handling wildlife (whether in the field or laboratory). Such information should accompany the individual when examination or treatment by a medical practitioner is needed. See ***Emergency Preparedness (Section 3.)*** for accident reporting forms.

Applicable local regulations regarding the documentation and reporting of workplace injuries should be consulted and followed.

Field supervisors should also maintain a record and pertinent product information of all immobilization drugs in their possession and as well as their usage.

Section 5.2.5c. Principles and Guidelines for Animal Care and Safety during Capture

All appropriate measures should be taken to minimize injury or harm to animals during capture and handling. Animals can sustain injuries or develop pathologic conditions during capture that could put their life in danger or decrease their chances of survival in the wild. Appropriate handling and restraint techniques should be used, and training in how to apply them should be provided to avoid injury to animals.

The investigator also must ensure that all workers fully understand and are trained in the techniques to be used for restraint and handling of wild animals. Improperly trained individuals or improperly applied techniques could harm the animal during capture and handling. Capture and handling can be accomplished using physical or chemical restraint or a combination of both.

Several factors must be considered to determine what type of restraint will be used on a specific project:

- Animal species and condition (sick, stressed, nursing, etc.)
- Safety for the staff to carry out the capture
- Animal safety
- Feasibility of accomplishing the capture's objective with the type of restraint
- Availability of drugs and specialized equipment to carry out the capture procedure
- Ability to protect, observe, and assist an animal until it has fully recovered after the procedure

Physical Restraint

Physical restraint may be most appropriate for some species and/or for short procedures. Animal handlers must ensure that physical restraint is performed in such a way that the animal will not suffer excessive stress or any injury during the process.

Physical restraint should be accomplished with necessary PPE, including latex or nitrile gloves and specialized equipment such as leather gloves, nets, rabies-poles, as needed for the species being handled. The capture team must be capable of correctly using and operating such equipment to avoid animal and human injuries during capture.

At a minimum, disposable gloves must be worn during handling and during the operation of specialized equipment. Researchers must be trained and capable of using and operating all equipment used for capture to avoid injuring the animal. When using leather gloves to restrain an animal, the operator must ensure that excessive pressure is not applied to avoid suffocating the animal. When using nets to capture wild animals, removal from the net should occur immediately to avoid further entanglement and possible fatal and non-fatal injuries to the animal.

The risk of causing trauma to an animal when using a snare or rabies-pole is high, thus the benefits should outweigh the risks when considering their use. If using a snare-pole, it is important that the snare be placed around the neck AND one of the front legs of the animal to prevent the risk of asphyxiation.

In certain cases and with species that are vulnerable to stress, chemical restraint may be more appropriate.

<u>Chemical Restraint</u>

Chemical restraint should be considered when physical restraint is not safe for either the personnel or the animal being captured. Chemical restraint should be performed by veterinary professional with previous experience and specialized training in the use of anesthetic drugs and field anesthesia procedures.

The following considerations should be taken into account when deciding to chemically restrain an animal:

Considerations in Drug Selection

- Investigators should be familiar with the different drugs and drug combinations that can be used to safely capture a particular wild animal species.
- All drugs have intrinsic cardiovascular effects. The supervising veterinarian must be familiar with these effects and how to respond to any complications caused by these effects.
- Supervising veterinarians must be aware of any contraindication for the use of particular drugs on target species under the existing conditions.
- Investigators and supervising veterinarians should be aware of the availability (or lack of) of certain anesthetic drugs (and their reversal agents) and the regulations in place to import them into the country.

Considerations in Drug Administration

- During field captures, drugs that are often administered by injection can be administered via hand injection, pole-syringe (manual, spring-loaded) or darts (dartgun or blow darts).

- Staff should be familiar with the use of equipment (darts, pole-syringes) to avoid harming the animal during drug administration and immobilization.
- When hand injection is elected, the correct size needle should be used to avoid excessive trauma to the muscle or a penetrating wound in vital organs.
- Staff using darts to administer drugs should be trained in the use of the equipment required for the species and field setting. In general, only animals >15kg should be darted with powered dart-delivery equipment (i.e. pistols, guns).
- Darts have the potential to cause trauma if projected with excessive force or injected into a non-targeted area. Practice is the best way to assure that appropriate force is used when darting an animal.

Sites of Injection

- Anesthetic drugs should be injected into large muscle masses.
- Excessive force should be avoided when firing a dart as this could result in broken bones or perforation.
- Darts placed into the abdominal or thoracic areas are potentially fatal for the animal. Personnel should take great care to avoid placing a dart into one of these body areas.

Monitoring Anesthesia

- Anesthesia should be kept as brief as possible to minimize risks.
- When an animal is under chemical restraint, it should be monitored constantly to detect possible complications during anesthesia.
- An anesthetized animal should never be left unattended.
- Anesthetized animals cannot regulate their body temperature. Measures should be taken to prevent hyper- or hypothermia.
- Monitoring should include at least temperature, heart rate, respiratory rate and partial saturation of oxygen using a portable pulse-oximeter.
- The staff should be trained to respond properly to any emergency or complication occurring during anesthesia (e.g., how to treat hyperthermia), and appropriate reversal/ termination of anesthesia to avoid complications.

Recovery

- Animals must be released only once fully recovered from anesthesia .
- Animals should be allowed to recover in safe areas, away from hazards and areas where potential predators or aggressive con-specifics may be present.
- Once released, the animal must be observed for as long as is required to ensure it is awake, alert, and active
- If the animal suffers an injury during capture, the injuries should be treated appropriately before releasing the animal.
- If the injury sustained is life-threatening or will render the animal incapable of surviving in the wild, humane euthanasia should be considered. In some cases placing injured animals in rehabilitation facilities might be an option.

Note: A wealth of knowledge and expertise, as well as additional training materials are available within the PREDICT consortium. Staff should raise any concerns or questions regarding procedures for safe and ethical animal capture and handling to their partner surveillance leads. Regular surveillance team operational meetings are held to address questions or concerns encountered during surveillance activities and facilitate cross-partner distribution of knowledge and best practices in safe animal capture and handling.

Section 5.2.5d. Safe Animal Capture Guide Checklist

***Procedures Checklist*: Personnel working in the field with wild animals should follow these basic personal procedures:**

- ☐ Coordinators should provide all personnel a "Useful Contacts" list with address and numbers of local medical and emergency response services.
- ☐ Researchers working with wild mammals should consider pre-exposure rabies vaccination.
- ☐ Rabies vaccination should be given to personnel who routinely handle high-risk species in the wild (bats, raccoons, etc).
- ☐ Researchers and their assistants should also consider vaccination against tetanus in those situations where exposure to this pathogen is possible.
- ☐ Individuals who are exposed to potential vectors of rabies (e.g. animals with neurological signs) should immediately report the exposure to medical authorities and the supervisor.
- ☐ All animal tissues, fluids, and excrement should be handled so that the potential for human contact is minimized.
- ☐ Staff should thoroughly wash and/or sanitize hands and any other contaminated skin surfaces with a germicidal skin cleanser immediately after handling wild animals or their samples.
- ☐ All personnel handling wild animals should practice good hygiene and avoid rubbing their eyes after animal handling.
- ☐ Appropriate planning and specific precautions (trained staff, equipment and tools in good working condition, PPE, etc.) should be taken in order to prevent injuries from bites, scratches and skin punctures from wild animals. Even minor wounds or scrapes may become infected and can potentially result in disease transmission.
- ☐ If an injury occurs, clean the wound with a disinfectant and immediately contact a coordinatior/supervisor.
- ☐ Where there is a risk from aerosolized pathogens from saliva, feces or urine, protective gear such as gloves, eye protection, respiratory protection (masks, face-shields or respirators), foot protection and protective clothing should be used as necessary.
- ☐ Researchers should always wear gloves and facemask when handling sick or dead animals.
- ☐ Personnel performing post-mortem examinations in the field should wear at least a plastic apron, gloves and facemask or goggles.
- ☐ After any post-mortem examination is performed, staff should wash and disinfect hands and any other contaminated skin surface.
- ☐ All contaminated equipment should be cleaned and disinfected immediately after use while still wearing the appropriate PPE. Disposable used equipment must be adequately disposed of on site (i.e. buried, burnt, etc).
- ☐ All drug containers, needles, scalpel blades, suture needles and other sharp instruments should be used and disposed of in a manner that prevents accidental human injury.
- ☐ Physical restraint of wild animals should be kept as brief as possible.
- ☐ Care should be exercised when using equipment such as nets, gloves, rabies-pole, etc to capture wild animals.
- ☐ Staff should be familiar with dart equipment, sites of injection and drugs when chemical restraint is elected.
- ☐ Anesthesia monitoring equipment and emergency drugs must be available and staff should be familiar with their use.
- ☐ Staff will make sure each animal is fully recovered from anesthesia prior to release.
- ☐ A list of the equipment and supplies needed to correctly implement the recommendations of this Safety Guide for Animal Capture for Sampling is available and checked prior to departing for the field.

Checklist for Supplies for Animal Capture Activities

Check as appropriate:

PPE

- ☐ Nitrile (recommended) gloves
- ☐ Leather or kevlar gloves
- ☐ Face-mask
- ☐ Respirator
- ☐ Goggles
- ☐ Face-shield
- ☐ Disposable (Tyvek) suit
- ☐ Sharp-container
- ☐ Closed-toed shoes

Monitoring

- ☐ Thermometer
- ☐ Stethoscope
- ☐ Stopwatch or other timing device
- ☐ Pulse oxymeter with probes
- ☐ Penlight
- ☐ Warm-water bottles (to prevent hypothermia)
- ☐ Buckets or water bottles (to prevent hyperthermia)

Immobilization equipment

- ☐ Dart equipment (rifle/pistol/blowpipe, CO_2 or powder cartridges, and dart protectors)
- ☐ Drugs (sedatives, tranquilizers, anesthetic drugs, reversals or antagonists)
- ☐ Calculator to use for calculating drug dosages
- ☐ Darts and dart needles
- ☐ Ropes/hobbles
- ☐ Nets
- ☐ Pole-syringe
- ☐ Cargo-net
- ☐ Blindfold
- ☐ Ear-plugs
- ☐ Carrying bags
- ☐ Syringes and needles
- ☐ Towels
- ☐ Snare-pole

Emergency

- ☐ Emergency medications (doxapram/ atropine/epinephrine/diazepam)
- ☐ IV catheters
- ☐ Fluids (NaCl, lactated Ringer's, Dextrose)
- ☐ IV administration set
- ☐ Antibiotics, disinfectants
- ☐ Tongue swabs
- ☐ Vet wrap and tape
- ☐ Flashlight
- ☐ Minor surgery (and suture) pack
- ☐ Euthanasia solution
- ☐ Alcohol (to treat hyperthermia)
- ☐ Tissue glue or super-glue
- ☐ Blanket/towel (to help treat hypothermia)

☐ Cold pack/hot pack
☐ Laringoscope, tracheal tubes, ambu bag

Recovery and release
☐ Crates/containers in which to place animal during recovery
☐ Binoculars

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Section 5.2.12. Bushmeat Sampling Methods

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Objective: Guidance on how to collect biological samples from hunted wildlife and wildlife byproducts in the context of the PREDICT project.

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The authors assert that animal capture and sampling should always occur in compliance with all applicable laws and regulations and should only be undertaken after securing all necessary permits and approvals, including ethical approvals.

For more information about the contents of this guide, please contact predict@ucdavis.edu.

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Table of Contents: Bushmeat

Section 5.2.12a. Confirmation of Knowledge

When you are familiar with the information in this guide, take the PREDICT quiz ***Section 8.4.11 Bushmeat Sampling***.

Section 5.2.12b. Brief Overview of PPE

Minimum PPE Required for Bushmeat Sampling

The minimum PPE for sampling small carnivores includes:

- Double gloves
- Protective glasses
- N95 facemask for self-protection and to avoid contaminating samples

Note: Wear appropriate PPE *according to species and pathogen-associated risk level.*

(See the ***Biosafety and PPE Guide (Section 4.)*** for detailed instructions regarding PPE Use)

Section 5.2.12c. Bushmeat Sample Collection

Samples to Collect

Duplicate specimens are to be collected from each animal (if feasible). If only one sample can be collected, then place into VTM.

1) Blood
   a) **Fresh kill**: collect whole blood and serum.
      i) **Whole blood: Collect as much blood as possible. Cardiac puncture is recommended.** Collect whole blood into 1 lavender top tube containing EDTA. Transfer a max of 500 µL of whole blood to cryovial containing 500 µL VTM and another 500 µL of whole blood to cryovial containing 500 µL Trizol. Freeze in liquid nitrogen or -80°C freezer.
      ii) **Serum**: collect blood in at least 1 serum separator tube. Allow blood to clot and store a minimum of 2 x 0.5mL serum aliquots, frozen without media.

Note: If blood volume recovered is too small to collect two blood tubes (for whole blood and serum), collect serum and save remaining clot in 500 µL VTM after serum separation.

   b) **Carcass**: collect blood clot. Place in at least one cryovial containing 500 µL VTM, and freeze in liquid nitrogen or -80°C freezer.
2) **Swabs** (if fresh kill – x2 of each swab type): Collect 2 oral and 2 rectal swabs placing 1 of each sample into 500 µL VTM and Trizol, respectively. Rectal swabs can be moistened with sterile saline prior to animal sampling.

3) **Tissue:** Collect three, adjacent, approximately 200mg (pea-sized) samples from each of the following organs:

- Adrenal
- Colon
- Heart
- Liver
- Lymph node
- Ovary
- Testes
- Cecum
- Duodenum
- Kidney
- Lung
- Spleen
- Pancreas
- Other, if required

One specimen should be frozen in 500 µL VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).

Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

If there is no **short-term** access (i.e., within 24 hours) to cold chain such as in an emergency situation then samples can be collected in 500 µl of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

- 1 day at 37 °C (i.e. ambient temp)
- 1 week in the refrigerator
- Within one week freeze at -80 °C for storage until analysis

Section 5.2.12d. Bushmeat Sample Collection Methods

Sample Collection Technique

1. Ensure all sample collection tubes or vials are pre-labeled with appropriate information pertaining to sample ID (unique sample ID, or barcode and/or date).
2. **Wear appropriate PPE** according to species and pathogen-associated risk level (see above for minimum requirements).
3. Sample methods:
    a. Use sterile, disposable sample collection utensils (tweezers/scalpels/needle and syringe) or wipe and flame with ethanol or isopropyl alcohol any metal instruments (e.g. scissors and tweezers) before collecting each sample type.
    b. **For whole blood and serum** (fresh kill only):
        i. Label vacutainer and prop tube upright in tube holder.
        ii. If possible, perform cardiac puncture (laterally between ribs or longitudinally under sternum) using 3 ml or 5 ml syringe and adequate (largest possible for

size of species) size needle to reach heart and draw blood (e.g. 19G for larger animal) without opening the carcass. Alternatively, open thoracic cavity to reach the heart.

   iii. Transfer blood (retaining ~1 ml in the syringe if volume permits) from syringe to a serum separator or red top vacutainer by disposing of the needle to the sharps box, and uncapping the vacutainer. Do not contaminate outside of blood tube with blood (If this occurs lightly clean outside of tube with ethanol-moistened gauze prior to moving on). Place labeled vacutainer in rack in shade for up to 2 hours before following instructions below on "Blood clot".
   iv. Transfer up to 500 µL of the blood remaining in the syringe to a cryotube with 500 µL of VTM and an additional 500 µL of remaining blood to a cryotube with 500 µL of Trizol (maximum final ratio of 1:1 in both cases).

c. **For blood clot** (carcass where collection of whole blood is not feasible):
   i. Using a sterile scalpel blade or forceps, collect blood clot ensuring no contamination from the external environment. Blood clot should be placed directly into 500 µL VTM and frozen.
d. **For swabs (fresh kill only):** Using sterile polyester or Dacron-tipped (aluminum or plastic shaft – not wooden) swabs, collect 2 oral and 2 rectal swabs. Rectal swabs can be moistened with sterile saline prior to animal sampling. Place one oral and one rectal swab in separate cryovials filled with 500 µL VTM. Place one oral and one rectal swab in separate 2 ml cryovials with filled with 500 µL Trizol. After placement into the tubes, cut swab tips (with ethanol-flamed scissors) on the shaft as close to the swab tip without touching/contaminating it. Scissors should be wiped with ethanol or isopropyl alcohol and flamed between each sample. Alternatively, snap swab shafts above the tip. After closing tubes, mix each tube well. Sealed, labeled vials with samples are to be immediately stored in liquid nitrogen (dry shipper or dewar) until transfer to -80˚C freezer.
e. **For muscle tissue:** Using a sterile scalpel blade, dissect beneath the exposed surface to take three ~0.5 cm^3 (small pea-sized) samples of muscle tissue ensuring no contamination. Take muscle samples from most fresh area available (raw tissue preferable). Place one sample in a labeled cryovial with 500 µL VTM and recap, and place another in a labeled cryovial with 1 mL Trizol and recap. Store immediately in liquid nitrogen (dry shipper or dewar) until transfer to -80˚C freezer. Place third sample in labeled jar or vial with 10% buffered formalin at a volume of fixative 10 times the volume of the tissue, and store at room temperature.
f. **For organ tissue:** Using sterile/clean scalpel blade, take three 0.5 cm^3 samples of each organ tissue (see recommended list of organs above), ensuring no contamination from external environment. Organ samples should each be placed in individual, labeled cryovials. Place one sample in a cryovial with 500 µL VTM and another sample in a cryovial with 1 mL Trizol, and freeze samples at -80°C (or liquid nitrogen in the field). Place third tissue sample in a labeled jar or vial with 10%

neutral buffered formalin at a volume of fixative 10 times the volume of the tissue, and store at room temperature.

Additional sampling considerations:
In many bushmeat market or hunter-killed sampling situations, it may not be acceptable to traders for you to take organ samples. Remember that under PREDICT ethical guidelines, you CANNOT pay or trade anything for the samples. If allowed, intestinal/lymph node samples can often easily be obtained by inserting long hemostats into rectum and pulling out a sample of colon tissue. If the animal is to be butchered, you may also ask the owner/hunter to cut small samples of liver, lung, small intestine, large intestine, spleen, and kidneys. From these hunter samples, collect a small part of each organ tissue (~0.5 cm^3) while maintaining sterility to the extent possible (i.e. avoiding surface of original hunter-taken tissue and asking the trader to clean her/his knife between cuttings of samples of various organs). Remember that (legal or not) bushmeat is intended for human consumption so, during sampling be very careful not to contaminate carcasses with hazardous chemicals (e.g., Trizol or formalin) or to touch bushmeat with potentially contaminated gloved hands or non-sterile utensils.

The researcher must consider quality of specimens and the pathogens of interest when deciding whether or not to sample a carcass for pathogens. Tissue from animals that have been smoked, dried, or dead longer than 24 hours are much less likely to harbor live pathogens or detectable RNA viruses, and are more likely to contain contaminating agents and bacterial overgrowths. Other pathogens, such as DNA viruses, may be detectable in tissues for an extended period of time. Most types of tissue (including skin or hair) can be used for genetic analysis (species identification), even from specimens that are of lesser quality (dried, processed, etc.).

Required sample storage conditions:
- Store all collected specimens immediately in liquid nitrogen or -80 °C freezer.
- Keep all samples frozen in liquid nitrogen in a dry shipper or dewar until transfer to -80˚C freezer for long-term storage.
- Do not allow samples to thaw once frozen.
- Tissues in buffered formalin must be kept at room temperature.

Section 5.2.12e. References

CDC and WHO (Centers for Disease Control and Prevention and World Health Organization). 1998. Infection Control for Viral Haemorrhagic Fevers in the African Health Care Setting. Atlanta, Centers for Disease Control and Prevention, pp. 198.

WCS Field Veterinary Program 2006. Bushmeat-Handling Protocol for Ecoguards, Congo-Gabon.

Section 5.2.6. Non-Human Primate Sampling Methods

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Objective: To safely collect biological samples from non-human primates.

This document was made possible by the generous support of the American people through the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT program. It was drafted to support activities conducted under PREDICT and is intended for an audience of qualified professionals trained in standard, associated best practices. This guide is not intended for use by untrained individuals.

The authors assert that animal capture and sampling should always occur in compliance with all applicable laws and regulations and should only be undertaken after securing all necessary permits and approvals, including ethical approvals.

For more information about the contents of this guide, please contact predict@ucdavis.edu.

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Table of Contents: Non-Human Primate (NHP) Sampling

Section 5.2.6a. Confirmation of Knowledge

When you are familiar with the information in this Guide, take the PREDICT quiz ***Section 8.4.5. Non-Human Primate Sampling***.

Section 5.2.6b. Brief Overview of PPE

Minimum PPE Required for Handling Live, Dead, or Samples of NHP

The minimum PPE for NHP sampling includes:

1. Eye protection (goggles or face shields)
2. N95 (or better) respirator
3. Long clothing/tyvek suits (Duct tape can be wrapped around the overlapping tyvek suit and gloves at the wrist to avoid skin exposure)
4. Nitrile gloves (double gloving is preferred, especially if sampling dead NHP)
5. In the rare cases where it is acceptable (see below), anyone hand-restraining NHP for sampling should wear disinfected[1], heavy-duty leather (or similar) gloves to protect against bites
6. In order to protect both human handlers and sampled NHP, all personnel handling NHP should be tuberculosis (TB) tested beforehand as described below

Macaque Handling

Due to the risk of infection with *Cercopithecine herpes 1* ('B virus'), which can be fatal in humans, handling macaques (or other potential B virus carriers such as other NHP in close contact with macaques) requires special preparation. When handling macaques, it is imperative that before animals are handled all precautions are taken to minimize the risk of exposure to B virus and to minimize the risk of infection in the event of an accidental exposure. Please note that human to macaque transmission of herpesviruses may also occur. Protective measures include:

- Wearing a full-face shield (not just goggles) along with an N95 (or better) respirator.
- Having sufficient and immediately available eyewash (1 liter of saline if working in remote location) for a 15-minute continuous flush of any exposed mucous membranes.
- Having water and detergent soap (chlorhexidine or povidone-iodine) immediately available and in sufficient quantity to allow a 15 minute scrub of any exposed skin.
- Preferably also having freshly prepared 0.25% hypochlorite/Dakin's solution (1:20 dilution of household bleach) for initial wash of skin - but NOT mucous membranes.

[1] Because they are porous, leather gloves cannot easily be disinfected. Spraying, wiping, or soaking in the best available disinfectants (e.g., 10% bleach) and allowing to sit or dry for >10 minutes can help destroy many potential pathogens. Likewise, wearing and changing over-sized disposable gloves over protective leather gloves can help to minimize cross contamination between handled animals. More easily cleaned protective gloves made from synthetic materials (heavy duty nitrile, Kevlar) can also be used. Some PREDICT teams have had good success with Hexarmor Hercules 400R6E gloves.

- Keeping extra swabs, viral culture media, and serosampling materials available for post-exposure sampling of handler and macaque.
- Carrying medical alert cards.
- Consider having a cage ready for short term (2-3 week) captivity of suspect macaques for post-exposure sampling in the event of accidental exposure.

First Aid Guidance for a Bite, Scratch, Needlestick, or Facial Splash

The injured person must notify other research staff and work must stop immediately (with the possible exception of other workers ensuring the safety and containment of any live animals).

All NHP - Any bite, scratch, or needlestick site should be immediately washed well with soap and water for a full 5 minutes and then with betadine (povidone-iodine) or benzalkonium chloride (if available and especially if rabies virus exposure is suspected).

Macaques (or other possible B virus carriers) - Any possible exposure to B virus is potentially life threatening and must *immediately* trigger activation of the B Virus Emergency Exposure Protocol detailed in ***Section 5.2.6g. Appendix II. B Virus Exposure Emergency Protocol***.

Suspect Ebola cases (e.g., ape carcasses) - Any possible exposure to Ebola virus is potentially life-threatening and should immediately trigger activation of the Ebola Virus Emergency Exposure Protocol detailed in ***Section 5.2.6h. Appendix III. Ebola Virus Exposure Emergency Protocol***.

Section 5.2.6c. Special Considerations for Handling NHP

***Note**: This training guide supplements the Safe Animal Capture and Sampling (Section 5.2.5), which contains general information on working with wildlife species. This training guide also complements information in the Bushmeat Sampling Methods (Section 5.2.12.).*

Handling NHP involves a number of special considerations.

1. Regardless of their specific status (e.g., endangered, threatened, protected or not), NHP are often high-profile species that engender special attention. Anyone handling NHP should strictly adhere to all regulations and follow all protocols and guidelines.
2. All primate species, regardless of size, are capable of inflicting serious injuries to their handlers; particularly bite wounds. Unlike most other taxa, many NHP have grasping hands and feet and are likely to grab (and then bite) rather than scratch or push their handlers during procedures. Heavy-duty leather gloves should be worn by anyone handling conscious (unanesthetized or unsedated) NHP. Hand restraint is discouraged as a primary means of NHP immobilization. Chemical, rather than physical, restraint should be employed with a few exceptions. Hand restraint may be considered in rare instances when it can be done safely and without significant added stress or risk to the animal,

such as when handling infants, severely debilitated individuals, or during the process of chemically immobilizing very small NHP with hand injections.

3. NHP are typically very social animals and are likely to protect and defend other individuals in their group. Care must be taken, particularly during capture and immobilization, to protect against attacks, injuries, or disruptions from non-target individuals and especially from defensive adult males. Using visual blinds to hide activities and/or employing personnel fully dedicated to watching for aggressive or approaching animals can help minimize these risks.
4. Due to their size, considerable strength, and in some cases habituation to human visitors, great apes (and some larger monkeys) should be considered very dangerous. Even without aggressive intentions, field staff should be aware that great apes often grab, kick, strike, and drag humans for play and/or display behavior purposes.
5. If NHP need to be tracked for capture, or are opportunistically sampled as individuals or in low numbers, it may not be feasible or practical to set-up proper sampling stations as described below. In such situations, sampling station guidelines should be followed as closely as possible for both field collection sites and any later sample processing sites.
6. PREDICT personnel should already understand that due to their close genetic relationship to humans, NHP are considered to be more likely to share infectious agents (zoonoses) with humans. This means that they more likely to transmit infections *to* their human handlers, and they are also more susceptible to acquiring infections *from* their handlers.
   a. Proper use of PPE and related biosafety measures as described will help protect both handlers and the sampled NHP.
   b. To protect both staff and any handled NHP, all people working closely with NHP should be tuberculosis (TB) tested every 6 months with negative results documented and available before handling NHP. **Any staff suspected of being infected with TB must not work with NHP.** TB testing is typically done by intradermal tuberculin skin test (TST). Workers who have been vaccinated with BCG (Bacillus Calmette-Guerin, standard vaccine for many Europeans) should still be tested and the possibility of false positive results from vaccination needs to be discussed with their health care provider (see relevant information at: http://www.cdc.gov/tb/publications/factsheets/testing/diagnosis.htm). Personnel vaccinated with BCG and positive skin test should work with their health care provider to have additional confirmatory tests performed.
   c. To protect NHP from human infections, no persons with any current or recent (within a few days[2]) clinical signs of illness (coughing, sneezing, fever, diarrhea, rash, cold sores, etc.) should handle or have close contact (<5 m) with any NHP. It must be remembered, however, that many agents are infectious to other animals *before* the infected individual becomes clinically ill (or after recovery). Ideally, personnel working

[2] There are no distinct time rules because pathogen shedding depends on many host and pathogen-specific factors. Though infectivity can in some cases range up to many months after resolution of clinically apparent disease, in healthy adults most pathogens of concern here (e.g., respiratory viruses) are unlikely to be transmissible for more than a few days after recovering from illness.

regularly with NHP should participate in some level of an employee health program, and be up to date on all available vaccinations (especially measles, polio, hepatitis A, influenza(s), meningococcal meningitis, rabies, and tetanus). This helps to ensure their health and to protect their co-workers and any animals they may handle.

7. NHP are not typical sources of rabies virus transmission to humans, but like any mammal must be considered a risk, especially in areas where they might be regularly exposed to common, high-risk rabies reservoirs (e.g., domestic dogs in many countries). If there is any suspicion of rabies exposure (e.g., handler is bitten by or exposed to nervous tissues from a primate exhibiting neurologic signs), their physician should be contacted and post-exposure rabies vaccination should be obtained as soon as possible. Rabies symptoms in primates are variable (irritability, self-mutilation, paralysis, malaise).
8. NHP are also not typical sources of anthrax exposure in humans, but are known to suffer and even die from anthrax, including in atypical forest environments. Proper PPE use and appropriate disposal of suspect carcasses are the most effective measures of preventing anthrax exposure. For additional information see **http://www.bt.cdc.gov/agent/anthrax/**.
9. Two particularly important and dangerous pathogens that workers may be exposed to by handling NHP are Ebola virus and *Cercopithecine herpes*-1 (B virus). **EXPOSURE TO THESE PATHOGENS IS LIFE-THREATENING AND REQUIRES IMMEDIATE ACTION.**

   B virus- PREDICT staff are most likely to be exposed by handling live macaques, which should **always** be assumed to be infected with B virus, with or without any clinical signs. *Macaques with oral lesions (right) should be handled with **extreme** caution and only by highly trained staff, if they are handled at all.* Macaques shed the virus in their oral, gingival, and genital mucosa and transmission can occur via bites, scratches, percutaneous inoculation with infected materials (e.g., accidental needlestick), and mucosal splash exposure. There is risk of B virus exposure from macaque CNS (central nervous system) tissues and CSF (cerebrospinal fluid), but peripheral blood from macaques has not been known to cause infection in humans. To prevent exposure to B virus, workers must always follow all PPE procedures and the precautions outlined below. In the event of accidental exposure, workers must stop ***IMMEDIATELY*** and trigger the ***B Virus Emergency Exposure Protocol*** detailed in ***Section 5.2.6g. Appendix II.*** TIMING IS CRITICAL and an immediate action can be the difference between life and death. Additional information on B virus can be found here:

   http://www.cdc.gov/herpesbvirus/index.html
   http://www.cdc.gov/mmwr/preview/mmwrhtml/00015936.htm
   http://www2.gsu.edu/~wwwvir/index.html

Ebola virus (and related *Filoviruses*) - PREDICT staff are most likely to be exposed by handling dead African ape carcasses, including bushmeat. Transmission can occur through contact with infected tissues, secretions, and body fluids and can be prevented through proper use of PPE and related barrier techniques (see www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/ebola.htm or http://emedicine.medscape.com/article/216288-treatment for more detailed information). Extreme caution must be taken by anyone sampling cases where Ebola infection is suspected. In the event of accidental exposure to Ebola virus (e.g., needlestick injury, any direct contact of eyes, skin or mucous membranes with infected fluids) workers must stop immediately and follow the details in ***Section 5.2.6h. Appendix III. Ebola Virus Exposure Emergency Protocol***. Symptoms of Ebola and complications are treated as they appear. The following basic interventions, when used early, can significantly improve the chances of survival:

- Providing intravenous fluids (IV) and balancing electrolytes (body salts).
- Maintaining oxygen status and blood pressure.
- Treating other infections if they occur.

Recovery from Ebola depends on good supportive care and the patient's immune response (http://www.cdc.gov/vhf/ebola/treatment/).

Section 5.2.6d. Primate Sampling

Note*: Capturing, trapping, darting, and immobilizing NHP should only be performed by experienced and skilled staff and are not entirely covered in this document (Hughes, T. 2010).*

PREDICT partners are expected to have detailed capture/immobilization protocols (and recording sheets, monitoring sheets, etc.) for any target primate species. This sampling protocol assumes a starting point of either a safely immobilized or an already dead primate. A vervet monkey capture and anesthesia guide is provided in ***Section 5.2.6i. Appendix IV. PREDICT Vervet Monkey Capture and Anesthesia Guide***.

For the PREDICT project, post-capture processing will entail a number of sometimes concurrent activities. The main objectives during processing are:

1. Safeguard the health of all handlers and any live animals being processed.
2. Collect required sample data.
3. Collect required biological samples.
4. Collect supplemental data and samples.
5. Await animal recovery or dispose of carcass.
6. After recovery, release animals as close to their site of capture as possible and follow all other guidelines for release as stated in the PREDICT IACUC protocol.

In some cases, time constraints, anesthetic risk, inability to prolong immobilization, or other factors may necessitate prioritizing biological sample collection at the expense of collecting any physical measurements. At a minimum:

1. Obtain and record the animal's weight (kg) as this can be important for proper drug dosing or emergency interventions and to estimate the age category.
2. Conduct a cursory physical exam before sampling in order to note any lesions or major abnormalities.
3. If capture wounds are observed, treat as needed.

Sample Data Collection

Please refer to the required data collection templates for data to collect:

1. P2 Animal Data Collection Form
2. P2 Site Characterization Data Collection Form
3. P2 Specimen Data Collection Form

Note: *See* ***Section 5.2.3. General Data Collection Templates and Applications*** *for help downloading templates from EIDITH.*

Additional (Optimum) Data to Collect from NHP

Note: *The P2 data templates mentioned above are required to be filled in. Additional data and biometric measurements may be collected at the discretion of the sampling party.*

Ideally, the following additional data should be collected from any NHP that are processed:

- body mass (kg)
- age class (see below)
- sex (and possibly reproductive status if adult female)
- whole body photograph(s)
- identifying characteristic photographs
- morphometric measurements

Body mass: Body mass may be one of the first measurements taken in order to ensure proper drug dosages, etc. Being careful to monitor breathing, and depending on size, NHP should be weighed (kg) in bags, slings, or a suitable container using a calibrated hanging spring scale or, if they are small enough, a tabletop scale with or without a tray or other container. If large NHP exceed the limit of spring scales two or more scales can be linked (one hanging from the other) to distribute the weight. The total weight is the measure of both scales added together. Scales should be zeroed (checked to make sure they measure '0.0' units when empty) and any containers (bags, slings, trays, boxes) should be weighed beforehand and then both primate and container should be weighed together. Once the primate is removed from the container for sampling, the container should be re-weighed and subtracted from previous total. Alternatively, the weighing container can be tared so that the scale reads '0.0' units with the container, and then checked to verify it still measures exactly zero after the primate is removed. If scales are not available or accurate weights cannot be measured for any reason, a weight should still be estimated but the recording sheet *MUST note that it is an estimated and not a measured weight.*

Age class: If exact age is known (e.g., for habituated NHP) that should be recorded. Otherwise, for most primate species it will be possible to classify into one of the age classes in the table:

Age Class	Description
Neonate	Animal shows signs of being born within a few days.
Infant	Animal is unweaned and usually still clinging to mother and suckling.
Juvenile	Animal is mostly independent from mother, not yet adult-sized, and sexually immature.
Immature	Any individual not evidently sexually mature.
Subadult	Animal is fully independent, appears to be sexually mature, but not fully physically mature (e.g., less than full adult size).
Adult	Animal has secondary sexual characteristics, adult size, sexually mature.
Old Adult	Adult showing signs of age degeneration

Sex determination (species identification/examination): Based on morphology and unique characteristics, identify NHP to genus, and species (where possible) and sex. Sex determination for young individuals of many primate species is not always simple and photographs of genitalia should be taken, especially if there is any doubt. For female NHPs, note parity (e.g., presence offspring, evidence of previous lactation), also determine pregnancy status by gently palpating the abdomen (at least for small NHP), and determine lactation status by gently attempting to express milk from the teats (for larger NHP, milk samples can be collected in an empty cryovial and stored frozen). If dependent offspring are captured along with their mothers, they should not be removed from their mothers unless absolutely necessary (e.g., to prevent injury or if they are nearly independent/weaned) and then only for the minimal time required for sampling.

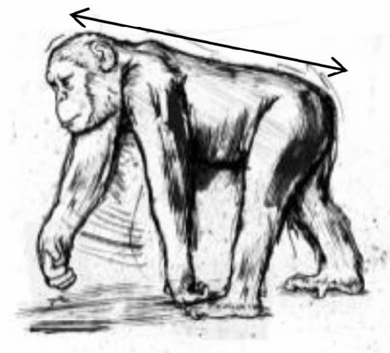
Photographs: At a minimum, the following digital photographs should be taken:

a. Anterior/ventral view of full body with arms at sides, preferably with identification card or sheet displaying unique identifying number.
b. Full anterior facial view.
c. Full lateral facial/head view.
d. Views of full upper and lower dentition (to help determine/verify age and sex).
e. Frontal/ventral view of fully exposed genitalia.

f. Views of any lesions (e.g., cuts, scratches), physical abnormalities (e.g., missing toes), or individually identifying marks or characteristics (e.g., healed scars, abnormal coloration, facial spots or wrinkles, etc.)

Body measurements: Time permitting, the biometrics (in cm or mm) should be recorded with the *minimum standard mammal measurements (all linear):*

a. Head and body length (measured dorsally and linearly from tip of nose to base of tail when head is stretched and aligned with back). Note: For many NHP (e.g., apes) this measure is adjusted to what is called "crown-rump" length that starts at the top of the head in order to produce the longest linear measurement (without wrapping over the head).
b. Tail length (from base to tip).
c. Hind foot length (heel to tip of longest toe- exclude nail and note which toe).
d. Tibia length ('knee to ankle').
e. Ear length: base of the notch below the ear opening (lower rim of external auditory canal) to the most distant point of the margin of the pinna.



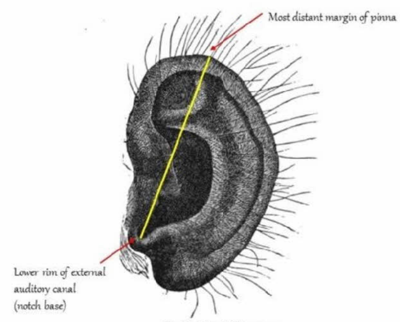


Fig. 5.—Ear of chimpanzee.
Anthropoid Apes, 1885

__Biological Sample Collection__

In addition to the standard PREDICT sampling and analyses, PREDICT partners are encouraged to collect additional samples and pursue routine diagnostics (e.g., blood counts and chemistries, urinalysis, etc.) where resources allow. Sample collecting for archival is also strongly recommended. Opportunities to collect biological samples and related health data from wild NHP are relatively uncommon and maximizing these opportunities can further advance wildlife health.

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two oral swabs** - one in 500 µL VTM and one in 500 µL Trizol
2. **Two rectal swabs/ fecal samples -** one swab in 500 µL VTM and one in 500 µL Trizol OR 0.5cc (pea size) feces in 500 µL VTM and 0.5cc (pea size) feces in 1 mL Trizol
3. **Two whole blood samples** - one with max of 500 µL of whole blood in 500 µL VTM and one with max of 500 µL of whole blood in 500 µL Trizol
4. **Two serum samples** - 2 x 500 µL aliquots, frozen without media
5. **Urogenital swab/urine samples** – one swab each in 500 µl VTM and 500 µl Trizol OR one 500 µL urine sample each in 500 µL VTM and in 500 µL Trizol

Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

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If there is no **short-term** access (i.e., within 24 hours) to cold chain such as in an emergency situation then samples can be collected in 500 µL of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

- 1 day at 37°C (i.e., ambient temp)
- 1 week in the refrigerator
- Within one week freeze at -80°C for storage until analysis

<u>Sample Collection from Live NHP</u>

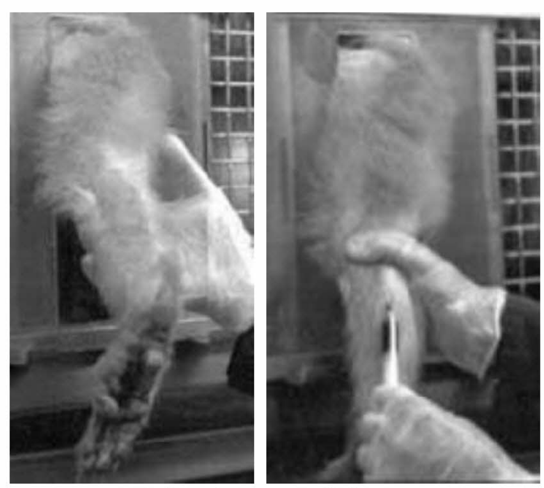
Live NHP should be chemically restrained during any invasive sample collection (e.g., blood collection). Two, preferably three, people are required for these manipulations: one person to safely restrain or position the primate, one to take samples, and a third to manage the tubes (e.g., unscrew the lids, hold them up to the sample taker, make sure the lids are replaced tightly and kept in order) and record sample data.

Blood Collection

Note*: At least one person present should have previous experience in primate venipuncture to avoid injury to the animal.* No more than 1 ml of blood per 100 g (= 10 ml/kg or 1%) of primate body weight should be collected at any one time.

Collection procedure

1. Select appropriate venipuncture site:
   - ***Forearm veins***- In larger species (e.g., apes), the cephalic, radial, median, and ulnar veins might be large enough for safe blood collection.
   - ***Femoral vein***- Best for small NHP and for large sample volumes. If the femoral artery (just lateral/anterior to the vein) is inadvertently pierced sampling can continue but extra effort must be made to apply post-collection pressure for at least 1 full minute to minimize hematoma formation.
   - ***Jugular vein***-This may be the only option in very small NHP and must be accessed carefully.
   - ***Caudal saphenous vein*** *(Figure right with laboratory macaque)*-With compression of the upper thigh or knee, this vein can be prominent and superficial, but often collapses during collection.



2. Select appropriate size needle and syringe (or vacutainer) for the size of the primate.
3. Disinfect the site with iodine solution or alcohol.
4. Collect sample.
5. ***Do not recap needle.***

6. Apply pressure to site of bleeding using a cotton ball or gauze pad until bleeding ceases (approximately 1 minute).
7. Process blood (see below).
8. Properly dispose of sharps and other biohazard materials immediately upon transfer of sample to collection vials and slides.

Blood processing
Place whole blood or blood clots in VTM and Trizol: If animals are large enough, collect whole blood into 1 lavender top tube containing EDTA and in 1 serum separator/serum-clotting factor tube (red top or tiger top) tube. From the EDTA tube, store 500 µl whole blood in a cryovial with 500 µl VTM and a second sample of 500 µl whole blood in a cryovial with 500 µl Trizol.

For serum, from the red top/tiger top tube, allow blood to clot and/or centrifuge. Use a plastic pipette to take 1 ml of serum and transfer into 2 cryovial tubes, 0.5 ml each. You can harvest additional serum for serum bank as appropriate. Transfer the remaining blood clots to separate cryovials. If the animal is not large enough to collect two blood tubes (for whole blood and serum), save the blood clot after serum separation. The blood clot should be placed in a cryovial with 500 µl VTM. Freeze all samples in liquid nitrogen in dry shipper or dewar and transfer to -80˚C freezer when possible.

(Optional) **Whole blood in EDTA:** If facilities are available to perform complete blood counts (CBCs) within 5 days, remaining whole blood in lavender top tubes can be refrigerated for analysis. Blood smears can also be prepared in the field.

Oral Swabs
Swabs in VTM and Trizol: Using two sterile, polyester-tipped swabs with a plastic shaft, rub the swab tip gently but thoroughly against the back of the primate's throat, saturating the swab with saliva. Place 1 swab in a cryovial filled with 500 µl VTM and use flame-sterilized scissors to cut the shaft of the swab above the tip. If using plastic shaft swabs when scissors aren't available, insert the swab to the bottom of the vial and then lift the tip and snap the plastic shaft of the swab on the edge of the cryovial. If the plastic shaft is snapped when the swab tip is resting on the bottom, the swab will be too long and the cryovial won't close. Place the other swab into 500 µl of Trizol in a cryovial, following the same procedures. After inserting the swab and closing the vial lid, shake each tube to mix the sample well. Store both cryovials in a liquid nitrogen dry shipper or dewar & transfer to -80˚C freezer when possible.

Feces
200 mg in VTM and Trizol: Collect either excreted feces or if primate is large enough *(> 1 kg)* use a gloved, lubricated (saline or medical lubricant) finger to collect feces directly from rectum. Place a ~200 mg (pea sized) sample of fresh feces in a cryovial with 500 µl VTM and another ~200 mg sample in a cryovial with 1 mL Trizol. Homogenize by shaking. *Freeze* in dry shipper or dewar with liquid nitrogen and transfer to -80˚C freezer when possible.

If feces are not available, collect **2 rectal swabs- 1 in VTM and 1 in Trizol.** Rectal swabs can be moistened with sterile saline prior to animal sampling. Gently insert one sterile swab tip at a time into the animal's rectum. [Note: DO NOT USE TRIZOL AS A LUBRICANT – IT IS HIGHLY IRRITATING TO TISSUE.] Place 1 swab in a cryovial filled with 500 µl of VTM using a flame-sterilized scissors to cut the shaft of the swab above the tip. Place the other swab into a cryovial with 500 µl Trizol. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80˚C freezer when possible.

Urine

Most NHP will urinate as a fear reaction prior to handling, but urine can sometimes be collected free catch or by bladder expression by trained personnel. Place 500 µl of urine in a cryovial with **500 µl VTM,** and another 500 µl of urine in a cryovial with **500 µl Trizol**. Store in dry shipper or dewar with liquid nitrogen and transfer to -80˚C freezer when possible.

(Optional) Ectoparasites (e.g., mites, lice, nits, fleas)

Collect any obvious ectoparasites (and hairs if necessary, e.g., for louse nits- pictured right) using forceps and place in labeled, appropriate sized container of **95% ethanol** and store at *room temperature.*

(Optional) Milk

If lactating females are handled, milk may be collected into cryovial(s) and stored frozen at -80°C. For basic analysis 0.5-2.0 ml is adequate and even small NHP (less than 500 g) can be milked to full evacuation one time and provide ~1 ml of milk without risking the health of their infants. Dependent offspring are typically best left with the nursing mothers and separation of nursing young prior to sampling should never be done strictly for the purpose of collecting milk.

<u>Non-Invasive Primate Saliva Sampling (Rope method)</u>

Field Collection Supplies:

- Six inch nylon oral swab ropes (Salimetrics, Inc)
- Nylon swab retrieval strings (if necessary)
- 5 ml compartmental swab storage tubes (Salimetrics, Inc)
- Cryovials
- Backpack/bag for concealing collection supplies
- Attractant (jam, bananas, juice, honey, etc.)
- Viral transport media
- Pipettor and tips (or disposable pipettors)
- Cooler bags and ice packs
- Field centrifuge
- Trash bags
- Spray bottle of disinfectant
- PPE (N95 masks, goggles, gloves)

Primate Groups for Sampling

Saliva sampling using distributed ropes is intended for use with semi-habituated primate species that will allow researchers to approach within a reasonable distance for sample collection. Precautions should be taken to avoid baiting primates into closer contact with local people or further habituating primate groups by limiting the number of times a single group is sampled and avoiding primates associating humans with distributing food.

Collecting Non-invasive Saliva Samples

1. While wearing gloves, dip 6-inch nylon swab ropes (Salimetrics, Inc) into an appropriate attractant (juice, jam, crushed banana, etc.). For some species (i.e. baboons), disguising the ropes completely inside a banana is more effective.
2. Walk around and look for isolated individual primates that are out of sight of the rest of the primate group. Try to identify individuals that are in the lower canopy or on the ground. When deciding on an area to sample, make sure there are no more than three primates in your perspective sampling area. Distributing ropes where large numbers of primates are present can initiate aggression between individuals.
3. Observe the social structure of the primates present carefully. Throw the rope to the most dominant primate (either adult male if present, or the largest adult female present). Juveniles may get the treat after the adult has discarded it. If you want to sample a juvenile primate make sure they are out of sight of other individuals.
4. Throw the rope when no primates are watching you and continue walking so the primate does not associate you with the treat.
5. Watch the primate as they chew on the rope. Keep a reasonable distance to avoid disturbing them. Follow the primate until it discards the rope. Do not approach a dropped rope until the primate has left the area and is no longer watching you.
6. When collecting the sample, have a designated person wearing PPE (N95 mask, eye goggles, gloves) approach the sample. Compress the chewed nylon swab rope with a gloved hand into a swab collection tube (Salimetrics, Inc.). Pipette 1ml of viral transport media (VTM) over the compressed rope in the swab collection tube. Store tubes on ice packs.
7. Move to a different location within the site to collect the next sample so no primates begin to associate you with distributing food. If it is difficult to retrieve dropped ropes because they are lost in tree branches, a thin nylon string can by sewn onto distributed 6-inch ropes to aid with retrieval.
8. In the laboratory or field processing station, centrifuge ropes for 15 minutes at 3,000rpm to elute saliva/VTM into the bottom collection compartment. Pour or pipette the saliva/VTM into labeled cryovial tubes and store in freezer at -80 degrees C.

Sample Collection from Dead NHP

If animals are found dead or must be euthanized by trained personnel per acceptable guidelines (see ***Section 8.5.2. AVMA's Euthanasia Guidelines*** and ***Section 8.5.3. AAZV's Euthanasia Guidelines***) due to health or welfare reasons, necropsy samples may be taken. When full necropsies are performed, following the American Association of Zoo Veterinarians (AAZV) great ape necropsy protocol is recommended and can be adapted for all primate species (see ***Section 5.2.6j. Appendix V. Occupational Primate Disease Safety Guidelines for Zoological Institutions***).

Note*: properly following this extensive necropsy procedure and collecting and measuring all samples can require 4-6 hours per animal.*

If carcasses are not whole or are fairly decayed, see ***Section 5.2.12. Bushmeat Sampling Methods***. If bodies are relatively whole and fairly fresh then blood, organ tissues, urine and (optionally) external parasites may be collected as described below.

Post-Mortem Blood Collection

From recently dead animals, it may be possible to collect whole blood (often clotted) from the right side of the heart where the largest volume of blood is available. Collect all available blood into an appropriate size container (typically one or more blood tubes). Allow the tubes to sit undisturbed for at least 30 minutes, and then centrifuge at high speed (2000 x G for 20 minutes). Use a plastic pipette to take 1 ml of serum and transfer into 2 cryovial tubes, 0.5 ml each. Transfer the remaining blood clots to separate cryovials. Freeze all samples in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab. If a centrifuge is not available, allow the clots and cells to settle as much as possible and collect serum as above.

Tissue Collection

Collect three, adjacent, approximately 200 mg (pea-sized) samples of the following tissues:

- Adrenal
- Colon
- Heart
- Liver
- Lymph node
- Ovary
- Testes
- Cecum
- Duodenum
- Kidney
- Lung
- Spleen
- Pancreas
- Other, if required

One specimen should be frozen in 500 μL VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).

Urogenital Swab/Urine

A urine sample should be collected if the carcass contains an intact bladder holding uncontaminated urine. Ideally, the sample should be collected with a 3 ml syringe attached to a 25 to 22 gauge needle. Insert the needle through the bladder wall and use the syringe to withdraw a maximum of 1 ml of urine. Do NOT stabilize the bladder by placing your hand beneath it, as this will put you at risk for needle injury. If the bladder is contracted (appears grossly empty of urine), use a sterile blade to make a small incision in the bladder wall. Small amounts of urine might be present and possible to suction up with a needleless 1 ml syringe inserted through the open incision. Place 500 µl of urine in a cryovial with **500 µl VTM,** and another 500 µl of urine in a cryovial with **500 µl Trizol**. Alternatively, if urine is not available, two urogenital swabs can be taken, with one placed in **500 µl VTM** and one in **500 µl Trizol.** Swabs can be moistened with sterile saline prior to animal sampling. Store samples in a dry shipper or dewar with liquid nitrogen and transfer later to -80˚C freezer.

Section 5.2.6e References

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Section 5.2.6f. Appendix I. Field Inventory Checklist

General Field Supplies:

- ☐ Headlamps
- ☐ AAA batteries and AA batteries
- ☐ Leatherman/Pocket knife
- ☐ GPS unit to mark site coordinates (in decimal degrees)
- ☐ Binoculars
- ☐ Brightly colored flagging tape

Workstation materials:

- ☐ Drapes, sheets, blankets, tarps, towels, plastic sheeting, etc.
- ☐ Scale and sacks, harnesses, ropes for weighing
- ☐ Disinfectants and clean-up supplies
- ☐ Biohazard bags (or plain bags and biohazard stickers) and sealing tape
- ☐ Hard, coverable container for transporting biohazard bags (if necessary)
- ☐ Anesthetic or immobilization drugs, medications, vaccinations
- ☐ Anesthesia monitoring equipment (pulse oximeter, stethoscopes, thermometer, etc.)

Data Collection Supplies:

- ☐ Field Data Collection sheets (Site/Event, Animal, and Specimen sheets)
- ☐ Clipboard or other weather resistant writing surface
- ☐ Pens and permanent markers
- ☐ Digital camera and charger
- ☐ Blank index cards or paper to label animals in photos
- ☐ Printed labels
- ☐ Waterproof paper (for use in formalin specimen jars)

Personal Protective Equipment (PPE)

- ☐ N95 (or better) respirators and a small stapler* (enough for all team members plus extras)
- ☐ **Safety goggles or face shields (for every person handling monkeys). Use face shields if handling suspect B virus or Ebolavirus positive NHP.**
- ☐ Long clothing/tyvek suits
- ☐ Disposable nitrile gloves
- ☐ First Aid Kit (with soap and betadine for cleaning wounds)
- ☐ Thicker gloves for primate handlers (i.e., leather to be worn over the nitrile gloves) that can be disinfected and re-used (e.g., Hexarmor Hercules 400R6E)
- ☐ Emergency exposure kits for B virus or Ebola (if applicable)
- ☐ Working communications equipment (cell phone, satellite phone, etc.)
- ☐ **Emergency response plan** (see ***Section 3. Emergency Preparedness Guide***)

* A small stapler can be used to staple the elastic straps back onto the N95 mask if they snap off

Biological data and sample collection supplies:

- ☐ Ruler, tape measure, or calipers appropriate for the size of the animal
- ☐ Serum separator blood tubes
- ☐ EDTA blood tubes
- ☐ Appropriate gauge needles or butterfly needles for smaller primates
- ☐ Syringes: 1mL, 3, 6 or 12mL
- ☐ Gauze or cotton to apply iodine to blood collection site
- ☐ Iodine for preparing blood collection site
- ☐ Sterile polyester swabs with plastic shaft for oropharyngeal, nasal, and fecal swabs

- ☐ Sterile saline
- ☐ Cryovials for storing serum, blood clots, swabs, feces, etc.
    - "Cryovials" refers to plastic, internally threaded screw-top vials with a silicon O-ring to prevent leakage. NUNC or Corning brand are recommended
- ☐ Viral Transport Media (VTM) and Trizol for storing specimens
- ☐ 95% Ethanol for storing ectoparasites
- ☐ 1.5mL microcentrifuge tubes for storing ectoparasites
- ☐ Necropsy kit for post-mortem exam in case of accidental death
    - 21 gauge needles (for cardiocentesis)
    - 22 to 25 gauge needles (for urine collection)
    - 1 and 6 mL syringes (for cardiocentesis and urine collection)
    - Scalpel and surgical blades
    - Forceps
    - Sharp and blunt tip scissors
    - 75%-80% Ethanol in small screw-capped vials for storing forensic entomological specimens
- ☐ Small jars containing 10% buffered formalin for histopathology specimens

Sample processing and storage supplies:

- ☐ Tube racks or cryovial boxes for storing tubes
- ☐ Field centrifuge to spin blood to separate serum
- ☐ Sterile plastic pipettes (for aliquoting serum)
- ☐ Surgical blades for dividing blood clot
- ☐ Cold Storage Container - Cooler and ice packs for specimens during collection
- ☐ Charged dry shipper for specimen field storage
- ☐ Cryoboxes, canes, or nylon stockings for organizing specimens in dry shipper

Waste Disposal Supplies:

- ☐ Sharps container (for needles and pipette tips)
- ☐ Trash bags or containers that can be disinfected
- ☐ Virkon or similar disinfectant that kills viruses
- ☐ Spray bottle for disinfectant
- ☐ Portable waste incinerator or other biohazard disposal plan

Section 5.2.6g. Appendix II. B Virus Exposure Emergency Protocol

(Adapted from Cohen et al., 2002. Recommendations for Prevention of and Therapy for Exposure to B Virus (*Cercopithecine Herpesvirus* 1). Clinical Infectious Diseases, 35: 1191-203.)

FIRST AID ***MOST IMPORTANT STEP***

Mucous membrane exposure: flush eye or mucous membranes with sterile saline solution or water for 15 min (or 1 liter).

Skin exposure: Wash skin thoroughly with a solution containing detergent soap (e.g., chlorhexidine or povidone iodine) for 15 min. Consider washing skin with freshly prepared 0.25% hypochlorite solution, followed by detergent solution, for 10–15 min.

INITIAL EVALUATION

Exposed worker

- Assess the adequacy of cleansing; the health care provider should repeat cleansing.
- Determine and document the date, time, location, and description of the injury, and the type of fluid or tissue contacted.
- Evaluate general health (including medications) and determine when the last tetanus booster was received.
- Determine the need for post-exposure prophylaxis with antibiotics or rabies vaccine and immunoglobulin.

NHP (*partly intended for laboratory NHP*)

- Identify the monkey associated with the exposure, the species of that monkey, and the responsible veterinarian.
- Assess general health (including medications and involvement in past and present research studies).
- Evaluate prior serologic history (including infection with B virus or simian immunodeficiency virus).
- Consider confining monkey for further evaluation and testing.

EXAMINATION AND LABORATORY TESTING

Exposed worker

- Physical examination, especially evaluation of the site of the exposure and neurologic examination.
- Consider obtaining serum samples at baseline for serologic analysis (pair at 2-3 weeks).
- Consider culturing specimens from the wound site or exposed mucosa after cleaning.

NHP

- Examine the animal for mucosal lesions (e.g., vesicles, ulcers), conjunctivitis, etc.
- Consider culturing specimens from the lesions, conjunctiva, and buccal mucosa.
- Consider serologic testing for B virus (if the animal is not known to be seropositive) and follow-up paired sample at 2-3 weeks.

EDUCATION AND TREATMENT

- Counsel the patient regarding the significance of the injury.
- Provide the patient with information on the signs and symptoms of B virus infection.
- Ensure that the patient has a card (to carry in his or her wallet) that includes information on B virus and a phone number to call for advice in an emergency.
- Ensure that the patient's occupational health care provider and supervisor are notified.

- Review with the patient and his or her work supervisor the safety precautions in place at the time of injury.
- Schedule a follow-up appointment.

CONSIDER POST-EXPOSURE PROPHYLAXIS

Pros and cons of post-exposure prophylaxis for persons exposed to B virus:

Pros

- Initiation of acyclovir therapy within 24 h after exposure to B virus prevents death among animals.
- Initiation of acyclovir therapy within hours of exposure may prevent or modify symptomatic B virus disease.

Cons

- Infection with B virus is very rare relative to the number of possible exposures.
- There are no controlled studies that document the ability of immediate empirical therapy to prevent infection or symptomatic B virus infection in humans.
- Acyclovir therapy can suppress virus shedding and seroconversion, which may make diagnosis more difficult.

Recommendations for post-exposure prophylaxis for persons exposed to B virus.

Prophylaxis recommended:

- Skin exposure (with loss of skin integrity) or mucosal exposure (with or without injury) to a high-risk source (e.g., a macaque that is ill, immunocompromised, or known to be shedding virus or that has lesions compatible with B virus disease).
- Inadequately cleaned skin exposure (with loss of skin integrity) or mucosal exposure (with or without injury).
- Laceration of the head, neck, or torso.
- Deep puncture bite.
- Needlestick associated with tissue or fluid from the nervous system, lesions suspicious for B virus, eyelids, or mucosa.
- Puncture or laceration after exposure to objects (a) contaminated either with fluid from monkey oral or genital lesions or with nervous system tissues, or (b) known to contain B virus.
- A post-cleansing culture is positive for B virus.

Prophylaxis considered:

- Mucosal splash that has been adequately cleaned.
- Laceration (with loss of skin integrity) that has been adequately cleaned.
- Needlestick involving blood from an ill or immunocompromised macaque.
- Puncture or laceration occurring after exposure to (a) objects contaminated with body fluid (other than that from a lesion), or (b) potentially infected cell culture.

Prophylaxis not recommended:

- Skin exposure in which the skin remains intact.
- Exposure associated with non-macaque species of NHP.

Section 5.2.6h. Appendix III. Ebola Virus Exposure Emergency Protocol

(adapted from **http://www.cdc.gov/ncidod/dvrd/spb/mnpages/vhfmanual/section5.htm**)

Accidental needlestick injury: Assume any needlestick injury is a suspected contact for viral hemorrhagic fever (VHF) whether or not a break in the skin can be seen. If an accidental needlestick injury occurs, contact the health care provider and treat the exposure site.

1. Immerse the exposed site in 70% alcohol for 20 to 30 seconds, and wash with soap and clean water.
2. Flush the site in running water for 20 to 30 seconds.
3. If needed, cover with a dressing.
4. Report the incident to a supervisor or the physician-in-charge.

The purpose of notifying the physician-in-charge is:

- To identify what caused the problem.
- To take corrective action to solve the problem and prevent accidental transmission.
- To provide appropriate care for the possible case of VHF.

Remind the exposed worker that accidents do happen even when every precaution to prevent them has been taken. Reassure worker that reporting the accidental exposure will have no negative consequences. Explain that reporting the accidental exposure is essential for protecting themselves, their families, other health workers and patients.

Accidental contact with infectious body fluids: An accidental contact can occur if there is unprotected contact between infectious body fluids and broken skin or the mouth, nose or eye. For example, vomit may run under a glove, a primate might cough blood which runs into the health care worker's eye, or splashed blood may run underneath a health care worker's mask and get into the mouth. Treat any accidental contact as a suspected contact with VHF. As soon as the contact occurs:

1. Flush the area in the most appropriate manner with soap and clean water. If a splash occurs in the eye, flush it with clean water.
2. Leave the isolation area and remove the protective clothing as recommended.
3. Take a shower and put on street clothes.
4. Report the exposure to a supervisor or the physician-in-charge. Complete the necessary forms.

Follow up accidental exposures:

1. Monitor the condition of the exposed worker. Take a measured temperature two times per day.

If a fever occurs -- temperature is 38.5°C (101°F) or higher -- the worker should not do any work activities and should seek immediate medical attention. Treat as a suspected case of VHF if the worker's signs and symptoms meet the case definition.

Section 5.2.6i. Appendix IV. PREDICT Vervet Monkey Capture and Anesthesia Guide

Monkey Capture

Information in this guide is based on field training with Mountain Gorilla Veterinary Project/Rwanda PREDICT staff and the PREDICT Primate Capture Training Protocol by Chris Whittier.

1. Assess monkey sleeping, foraging, and movement patterns in a given location. Working with local scouts to monitor monkey troop movements can make trapping efforts much more efficient.
2. After assessing movement patterns, identify a trapping location. During initial capture efforts, morning and late afternoon (as monkeys were leaving and returning to sleeping areas) were effective trapping times.
3. Prepare the Tomahawk non-collapsible metal traps by securely baiting them with a fresh banana before placing them near the target monkey troop. A long piece of duct tape folded in half lengthwise makes an effective strip for tying the banana to the bottom of the cage. The banana should be positioned just behind the treadle, so that it doesn't interfere with the ability of the treadle to trigger the trap to close (Figure 1). Tying the banana to the cage with at least 2 loops of duct tape ensures that the monkey can't steal the banana without triggering the trap (Figure 2).

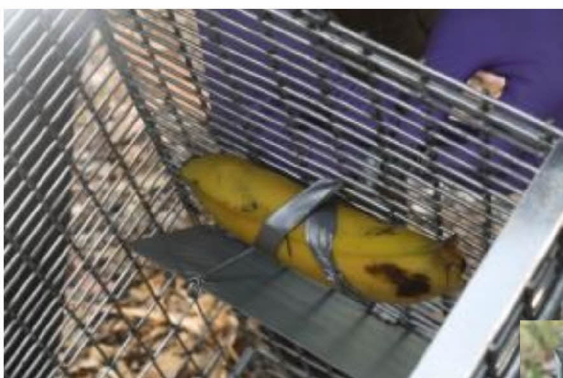

Figure 1: Tying a banana to the trap. ***Photo credit: PREDICT Tanzania Team***

Figure 2: Vervet Monkey triggering the trap while trying to remove a banana. ***Photo credit: PREDICT Tanzania Team***

4. Weigh the baited Tomahawk traps (in kg) using the appropriate spring scale. Set Tomahawk non-collapsible metal traps near the monkey troop. Place the traps on level ground, in a shaded location when possible. Capture staff should monitor the set traps from a distance to avoid disturbing the monkeys.
5. Once a monkey has been captured, weigh the trap with the monkey (in kg), and subtract the trap weight from the total weight to calculate the weight of the monkey. This weight is necessary for calculating doses of anesthetic drugs. Male vervet monkeys typically weigh 3-6.5kg. Females are usually 1.5-5 kg.
6. Following capture and weighing, traps containing monkeys should be removed to a sampling site and placed in a quiet, shaded location away from humans or other disturbances. Continue monitoring open traps to capture additional individuals. Monkeys in troops that have been captured previously may be extremely hesitant to enter traps.

Monkey Processing

Note: *Members of the country/field team should determine the specimens to be collected based on the interface sampled, the species of primates captured, feasibility, safety, and diagnostic requirements.*

1. Fill in site/event information on the data sheet and prepare animal and specimen data sheets for recording individual animal data. Use the GPS unit to collect site latitude/longitude in decimal degrees.
2. Set up a monkey processing station. Place leaves or other vegetation on the ground to insulate the anesthetized monkey from a cold or hot surface. Place a disposable plastic sheet or apron over the vegetation to create a sampling area that can be easily disinfected (Figure 3). Organize supplies in advance for easy access during sampling.

Figure 3: An anesthetized vervet monkey at the processing station with a PREDICT field team member identifying the femoral vein. ***Photo credit: PREDICT Tanzania Team***

3. All individuals handling monkeys and samples should wear appropriate PPE: N95 or better respirators, nitrile gloves, long clothing/tyvek suits, and safety glasses or face shields. Duct tape can be wrapped around the overlapping tyvek suit and gloves at the wrist to avoid skin exposure.
4. Using the weight of the captured monkey, calculate the appropriate anesthetic drug volumes. Vervet monkeys can be safely anesthetized using a combination of ketamine and medetomidine (Dormitor®) with the following doses:

   → 3.5 mg/kg of ketamine
   → 0.035 mg/kg of medetomidine‡
   ‡Dexmedetomidine may also be used at a dose of 0.0175 mg/kg

   Volume of drug needed (mL) = animal weight (kg) * safe and effective dose (mg/kg) ÷ drug concentration (mg/mL).
   Ketamine is commonly available in a concentration of 100mg/mL or 200mg/mL, and medetomidine is commonly 1mg/mL. Using these concentrations (100mg/mL for ketamine), a 5 kg monkey would require:

   5 kg * 3.5 mg/kg Ketamine ÷ 100mg/mL = 0.175 mL
   5 kg * 0.035 mg/kg Medetomidine ÷ 1 mg/mL = 0.175 mL

   The total volume of anesthetics (0.175 + 0.175 = 0.35 mL) can be drawn up in a single syringe for hand-injection of the monkey. A 22 or 25 gauge needle can be used to deliver the drugs.

5. Prepare the Atipamezole reversal (dose = 0.175 mg/kg) in a syringe and set aside in a cool place until sampling is complete. Note: when using 5mg/mL concentration of Atipamezole, the reversal volume is the same as the Medetomidine volume with 1mg/mL concentration of Medetomidine.
6. Once the drugs have been drawn up and combined in a single syringe, the monkey can be hand-injected through the bars of the trap. To reduce stress and to ensure that the full anesthetic dose is administered, the monkey must be confined to a small area of the trap where its movements are restricted. As a pilot method, the PREDICT Tanzania and Rwanda teams used 2 tools in combination to restrain the monkey in the cage. First, the comb (Figure 4, left) is inserted under the entry door of the trap to block the monkey from leaving. The entry door is then opened, and the plunger (Figure 4, right) is inserted. The comb is removed and then the plunger is pushed **gently** downward to confine the monkey to the bottom portion of the trap.


Figure 4: PREDICT tram inserting the comb just under the trap door so that the door can be opened without the monkey escaping (left). Anesthetic drugs are hand-injected through the bars at the bottom of the trap where the monkey is confined by the plunger tool (right). Photos courtesy of Mike Cranfield, Mountain Gorilla Veterinary Project.

7. Inject the anesthetic drugs intra-muscularly (target muscles of the thigh, shoulder or upper arm) and observe the monkey for initial signs of anesthesia: eyelids drooping, decreased movement, leaning against walls of trap, lying on floor of trap, unresponsive to stimuli. Signs of anesthesia should be visible within 5-10 minutes post-injection.
8. Once the monkey is anesthetized, carefully remove it from the trap. Open the sliding rear door of the trap a few inches to avoid escape by a partially anesthetized monkey. Quickly pull the monkey's arms behind its back (Figure 5). This safe strategy for holding small primates prevents a partially anesthetized or even fully aware monkey from biting the handler.

Figure 5: Dr. Mike Cranfield, from the Mountain Gorilla Veterinary Project, demonstrates the proper technique to restrain a vervet monkey. ***Photo credit: PREDICT Tanzania Team***

9. Place the anesthetized monkey on its back on the plastic sheet of the sampling station (see Figure 3). Ensure that the monkey has a clear airway, with unimpeded chest movements and unrestricted airflow in and out of the lungs. Assess and continually reassess the monkey's plane of anesthesia. During anesthesia, team members should monitor the monkey's breathing, heart rate, mucous membrane color, and capillary refill time. Rectal temperature and blood oxygen saturation (using a Pulse-oximeter) can also be assessed. Grip reflexes are among the first to return when a monkey is coming out of anesthesia.
10. Collect a **blood sample**. The femoral vein is the most reliable blood collection site in small primates. The femoral vein is found lateral and parallel to the femoral artery, which can be easily palpated in the inguinal region. Prepare the blood collection site by swabbing the area with iodine soaked gauze. Use a 25 gauge or smaller butterfly needle inserted at a roughly 45 degree angle to the skin to locate the femoral vein (Figure 6). Once blood is visible in the tubing of the butterfly needle, attach a syringe and apply gentle section. Avoid pulling the syringe too quickly, as too much vacuum pressure can collapse the vein.

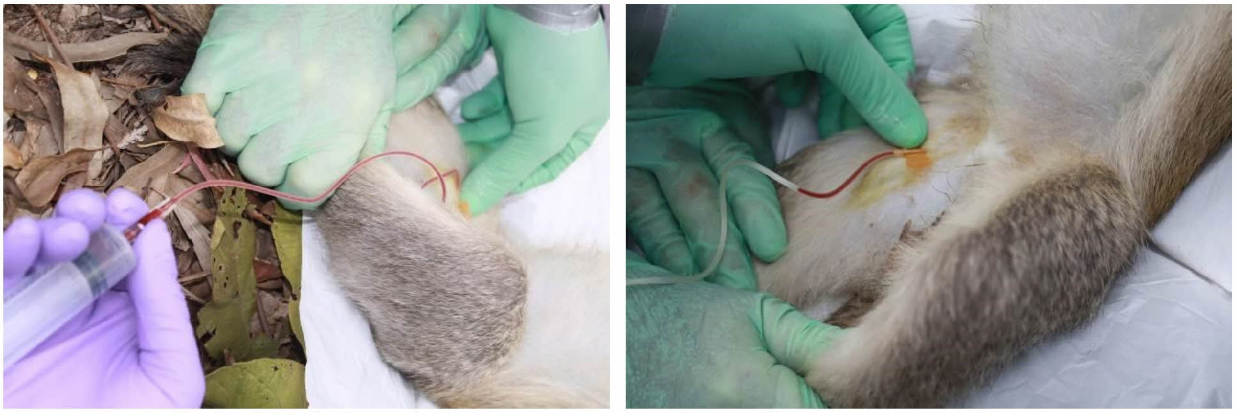

Figure 6: Blood collection from the femoral vein of a vervet monkey. ***Photo credit: PREDICT Tanzania Team***

11. Once the appropriate amount of blood has been collected, remove the needle and use gauze to apply pressure to the collection site for 1 minute to avoid hematoma formation. Store the collected blood in a serum separator tube or EDTA collection tube if whole blood is needed. Place the blood sample tubes in the cooler immediately after collection. **Do not recap the needle! Dispose of needle in the sharps container and the syringe in a biohazard or designated bag.**
12. Collect additional biological samples as described in the PREDICT biological sample collection protocol.
13. Before recovery, perform a physical exam of the captured monkey, noting any evidence of disease or injury. Capture related injuries can occur, and severity should be assessed prior to release. Small wounds and lacerations can be cleaned with iodine or betadine. More serious wounds may require veterinary intervention.
14. Mark the monkey with a temporary marker in a visible location such as the upper thigh (Figure 6) to avoid resampling the same animal when a troop is targeted for multiple days of capture efforts. The mark should wear off within one week.
15. Once sampling and physical examination are complete and the monkey has been anesthetized for a MINIMUM of 45 minutes, administer the Atipamezole reversal by intra-muscular injection. Earlier administration (<45 minutes post-induction) may result in ketamine-induced seizures. Place the monkey in a large dog kennel (or locally constructed container of similar size) in a quiet, shaded location away from humans to allow it to recover from anesthesia. It is extremely important to inspect and choose a safe release area away from hazards such as roads, people, or cliffs that could endanger the recently anesthetized monkey. Monitor the monkey regularly, and when it is fully aware and moving in a coordinated manner, open the door to allow it to return to its troop.

Figure 7: A vervet monkey with a temporary mark recovers from anesthesia. ***Photo by PREDICT Tanzania team***

Section 5.2.6j. Appendix V. Occupational Primate Disease Safety Guidelines for Zoological Institutions

(Appendix 3 – Standard Necropsy Report for Non-Human Primates Work Sheet; From http://www.aazv.org)

Appendix 3: Standard Necropsy Report for Non-Human Primates Work Sheet

Pathology #: ______________________ Species: __________________ Date: ______________________

Animal #/Name: _____________________ Sex: ___________________ Age (DOB): __________________

Date of death/euthanasia: ________________________ Time: _________________________(am/pm)

Method of euthanasia: ____

Time and date of necropsy: ____________________________ Duration of necropsy: ________________

Post mortem state: ________________________________ Nutritional state: _______________________

Pathologist or prosector and institution: ____

Gross diagnosis:

Abstract of clinical history:

Please check tissues submitted for histopathology.
External Examination (note evidence of trauma, exudates, diarrhea etc):
_______Hair coat:
_______Skin:
_______Scent glands:
_______Mammary glands and nipples:
_______Umbilicus (see neonatal/fetal protocol):
_______Subcutis (note: fat, edema, hemorrhage, parasites):
_______Mucous membranes (note: color, exudates):

Ocular or nasal exudate?:
_______Eyes and ears:
______External genitalia:
_______Oral cavity, cheek pouches and pharynx: Dentition (see attached dental form):
_______Tongue:

Musculoskeletal System (Note fractures, dislocations, malformations?):
______Bone growth plate (rib, distal femur, sternabra)
_______Muscles:
_______Bone marrow (femur):
_______Joints (note any exudates or arthritis):
_______Spinal column (examine ventral aspect when viscera removed)

Examination of the neck region:
_______Larynx:
_______Laryngeal air sac (see protocol for great apes):
_______Mandibular and parotid salivary glands:
_______Thyroids and parathyroids:
_______Cervical/cranial lymph nodes:
_______Esophagus:

Thoracic Cavity (Note any effusions, adhesions, or hemorrhage):
Note amount, color and any lesions in mediastinal and coronary fat:
_______Thymus:
_______Heart (see attached protocol):
_______Great vessels:
_______Trachea and bronchi:
_______Lungs:
_______Esophagus:
_______Lymph nodes:

Abdominal Cavity (Note any effusions, adhesions, or hemorrhage?):
Note amount, color or lesions in omental, mesenteric and perirenal fat:
_______Liver and gall bladder:
_______Stomach:
_______Pancreas:
_______Duodenum:
_______Jejunum:
_______Ileum:
_______Cecum and (in apes) appendix:
_______Colon and rectum:
_______Lymph nodes:
_______Kidneys and ureters:
_______Adrenals:
_______Gonads:
_______Uterus:
_______Bladder and urethra:
_______Male accessory sex glands (prostate and seminal vesicles):
_______Umbilical vessels, round ligaments of bladder in neonates:
_______Abdominal aorta and caudal vena cava:

Nervous System:
_______Meninges:
_______Brain:
_______Pituitary:
_______Trigeminal (gasserian) ganglia:
_______Spinal cord (please note to which lumbar segment the cord extends):
_______Brachial plexus and sciatic nerves:

Is there an identifiable pineal gland?

WEIGHTS AND MEASURMENTS (in grams, kilograms, and cm, please)

Body weight : ____

Lymphoid tissue:
R. axillary LN: __________________ L. axillary LN: ___________________________
R. inguinal LN: __________________ L. inguinal LN: ________________________
Jejunal LN: ____
Spleen: _________________________________ Thymus: _______________________

Abdominal Organs:
Liver: ___________________________
R. Kidney: ________________________________ L. Kidney: _______________________
R. Adrenal: ________________________________ L. Adrenal: __________________________
R. Ovary: _________________________________ L. Ovary: ___________________________________
Uterus: ____
Placenta (weight and measure disc(s)): ____

Thoracic Organs:
Heart wt: ____________________________ Thymus (above): ______________________________
Height: ______________________________ Circumference at coronary groove: _______________________
Left Vent. Thickness: ______________________ Rt. Vent. Thickness: ______________________________
Septum: ____
Lt. AV valve circ. __________________ Rt. AV valve circ. ___________________________________
Aortic valve circ. ____________________________ Pulmonary v. circ. _______________________
Rt. Lung: _______________________ L. Lung: ____

Other:
Brain: ______________________________ Tumors? ___________________________________
R. Testes (wt.): ___________________________ L. Testes: _______________________________
Length x dia: ____
Penis (length x diameter): ____

STANDARDIZED BODY MEASUREMENTS FOR NONHUMAN PRIMATES INCLUDING APES:

Crown rump length (linear)____

Crown rump length (curvalinear)_______________________________________

Cranial circumference (above brow ridge)____________________________

Length of head (tip of jaw to top of crest)________________________

Width of brow ridge____

Chest circumference (at nipples)____________________________________

Abdominal circumference (at umbilicus)______________________________

Left arm: Shoulder-elbow:____elbow-wrist:______

wrist-tip of middle finger:_______________________________ pollex:____

Right arm: Shoulder elbow:____elbow- wrist:_______

wrist-tip of middle finger:______________________________pollex:__________________________

Left leg: hip-knee:_____________________________knee-ankle:_____________________________

ankle-tip of big toe:_______________________________________heel-tip of big toe:______

hallux:____

Right leg: hip-knee:__________________________knee-ankle:______________

ankle-tip of big toe:___________________heel-tip of big toe:__

hallux:____

ANCILLARY DIAGNOSTICS (CHECK IF PERFORMED, GIVE RESULTS IF AVAILABLE, NOTE LOCATION IF STORED, OR TO WHOM SENT):

Cultures:
bacterial: fungal:
viral:

Heart blood:
serum:
filter paper blot:

Parasitology:
feces:
direct smears:
parasites:

Tissues fixed in 10% formalin (list tissues or specific lesions other than those checked above):
Tissue fixed for EM: ___________________________ Tissue frozen: ____________________________
Impression smears: ____
Comments:

NONHUMAN PRIMATE POST MORTEM EXAMINATION

Collection of tissues

Tissues to be fixed in 10% neutral buffered formalin should be less than 0.5 cm thick to ensure penetration of formalin for fixation.

Initial fixation should be in a volume of fixative 10 times the volume of the tissues. Agitation of the tissues during the first 24 hrs is helpful to prevent pieces from sticking together and inhibiting fixation. Once fixed tissues may be transferred to a smaller volume for shipment.

Labeling of specimens

If pieces are small or not readily recognizable (e.x. individual lymph nodes) they can be fixed in cassettes or embedding bags or wrapped in tissue paper labeled with pencil or indelible ink. Another alternative is to submit lymph nodes with attached identifiable tissue, e.x. axillary with brachial plexus, inguinal with skin, bronchial with bronchus, etc.

Sections from hollow viscera or skin can be stretched flat on paper (serosal side down) and allowed to adhere momentarily before being placed in formalin with the piece of paper. The paper can be labeled with the location from which the tissue came.

The formalin container should be labeled with the animals name or number, the age and sex, the date and location, and the name of the prosector.

Tissues to be preserved

From the skin submit at least one piece without lesions, a nipple and mammary gland tissue, scent gland, any lesions and subcutaneous or ectoparasites.

Axillary and or inguinal lymph nodes may be submitted whole from small animals and should be sectioned transversely through the hilus in large primates.

Mandibular, and/or parotid salivary glands should be sectioned to include lymph node with the former and ear canal with the latter. Thyroids, if it is a small primate, may be left attached to the larynx and submitted with the base of tongue, pharynx, esophagus as a block. In larger primates, take sections transversely through the thyroids trying to incorporate the parathyroids in the section.

Trachea and esophagus and laryngeal air sac sections may be submitted as a block.

Cervical lymph nodes may be submitted whole if small or sectioned transversely.

A single sternebra should be preserved as a source of bone marrow. A marrow touch imprint may be made from the cut sternebra and air dried for marrow cytology.

Section of thymus or anterior pericardium should be taken perpendicular to the front of the heart.

Heart: weigh and measure heart after opening but before sectioning. Please fix longitudinal sections of left and right ventricles with attached valves and atria in large animals and the whole heart opened and cleaned of blood clots in smaller animals. In tiny animals the heart may be fixed whole after cutting the tip off the apex.

Lungs: if possible inflate at least one lobe by instilling clean buffered formalin into the bronchus under slight pressure. Fix at least one lobe from each side and preferably samples from all lobes. In little animals the entire "pluck" may be fixed after perfusion and sampling for etiologic agents.

Gastrointestinal Track: Take sections of all levels of the GI track including: gastric cardia, fundus and pylorus (or presaccus, saccus, tubular stomach and pylorus in colobines); duodenum at the level of the bile duct with pancreas attached; anterior, middle and distal jejunum; ileum; ileocecocolic junction with attached nodes; cecum and (in apes) appendix; ascending, transverse and descending colon. Open loops of bowel to allow exposure of the mucosa and allow serosa to adhere momentarily to a piece of paper before placing both bowel section and paper in formalin; or gently inject formalin into closed loops.

Liver: Take sections from at least two lobes, one of which should include bile ducts and gall bladder.

Spleen: Make sure sections of spleen are very thin if the spleen is congested; formalin does not penetrate as far in very bloody tissues.

Mesenteric (jejunal) nodes: section transversely; colonic nodes may be left with colon sections.

Kidneys: Take sections from each kidney: Cut the left one longitudinally and the right one transversely so they will be identifiable (or label). Please make sure the sections extend from the capsule to the renal pelvis. Adrenals: small adrenals may be fixed whole but larger ones should be sectioned (left - longitudinal and right transversely) making sure to use a very sharp knife or new scalpel blade so as not to squash these very soft glands.

Bladder: sections should include fundus and trigone. Please make sure to include round ligaments (umbilical arteries) in neonates.

Male gonads and accessory sex glands: Section the prostate with the urethra and seminal vesicles transversely. Section testes transversely. If testes are being collected perimortem for sperm retrieval, try to arrange to take small sections before the gonads are manipulated.

Female reproductive organs: Fix the vulva, vagina, cervix, uterus and ovaries from small and medium sized primates as a block (after making a longitudinal slit to allow penetration of formalin). Rectum and bladder (opened) can also be included in this block. In somewhat larger animals make a longitudinal section through the entire track.

In great apes make transverse sections of each part of the track and the ovaries. (See reproductive track protocols from the contraception advisory group if animals are to be included in their database.)

CARDIAC EXAMINATION FOR GREAT APES (AND OTHER PRIMATES IN WHICH CARDIAC DISEASE IS PRESENT)

Examine heart in situ. Check for position, pericardial effusions or adhesions. Collect for culture or fluid analysis if present.
Remove heart and entire thoracic aorta with "pluck".

Examine heart again. Check the ligamentum (ductus) arteriosus for patency. Check position of great vessels. Open pulmonary arteries to check for thrombi.

Remove heart and thoracic aorta from the rest of the "pluck". Examine for presence of coronary fat. Examine external surfaces especially coronary vessels. Note relative filling of atria and state of contracture (diastole or systole at death) and general morphology. (The apex should be fairly pointed.)

Measure length from apex to top of atria. Measure circumference at base of atria (around coronary groove).

Open the heart:

Begin at the tip of the right auricle and open the atrium parallel to the coronary groove continuing into the vena cava. Remove blood clot and examine the AV valves. Cut into the right ventricle following the caudal aspect of the septum and continuing around the apex to the anterior side and out the pulmonary artery. Remove postmortem clots and examine inner surface. Open left atrium beginning at the auricle and continuing out the pulmonary vein. Remove any clots and examine valves. Open the left ventricle starting on the caudal aspect and following the septum as for the right ventricle. When you reach the anterior aspect, clear the lumen of blood and identify the aortic outflow. Continue the incision around the front of the heart and into the aorta, taking care to cut between the pulmonary artery and the auricle. Open the entire length of the thoracic aorta. Remove all postmortem clots. You may gently wash the heart in cool water or dilute formalin to better visualize the internal structures and valves. Sever the thoracic aorta from the heart just behind the brachiocephalic arteries. Examine intima and adventitia and section aorta for formalin. Sever the pulmonary vessel and vena cava close to the heart.
Weigh and measure the heart and record.

Measure thickness of right and left ventricular free walls and the septum. (On the left side, do not measure directly through a papillary muscle.)

Measure the circumference of the right and left AV valves and the aortic and pulmonary valves using a pliable measuring tape (or use a piece of string and measure the string on a straight ruler).

Take sections for histopathology:

Sections should include:
longitudinal sections of left and right ventricles AV valves and atria.

Sections of myocardium from left and right ventricles including coronary vessels. Sections of papillary muscles. Sections from the septum at the vase of the AV valves (area of conduction system).

In small animal like callitichids, you may fix the heart whole.

Fix the entire heart, if possible by immersion in 10% buffered formalin for more detailed examination by a cardiac pathologist.

Other vessels:
Make sure to examine the abdominal aorta, iliac arteries and popliteal arteries (frequent sites of aneurysms in humans).

Note the location and severity of fibrous plaques, fatty streaks and atherosclerotic plaques and presence of mineralization or thrombosis.

POSTMORTEM EXAMINATION OF NONHUMAN PRIMATE FETUSES AND NEONATES
External examination of the fetus:

Weigh the fetus and make body measurements.

Measure the placental disc(s) and weigh the placenta. Note umbilical length and vascular patterns on the placenta.

Note presence of hair, freshness of the carcass (if dam is dead, is the decomposition of the fetus consistent with that of the dam) and any evidence of meconium staining.
Internal examination of the fetus:

Follow the general nonhuman primate necropsy protocol.

Make sure to note whether ductus arteriosus and foramen ovale are patent. Note also whether the lungs are aerated and to what extent.

Note dentition / erupted teeth.

Identify umbilical vein and arteries and check for inflammation. Make sure to save umbilicus and round ligaments of the bladder (umbilical arteries) for histology.

Mae sure to save a growth plate (e.g. costochondral junction or distal femur) in formalin. Cultures: Take as many of the following as possible: Stomach content or swab of the mucosa; lung; spleen or liver; placental disc and extra-placental membranes. Do both aerobic and anaerobic cultures if possible.

POST MORTEM EXAMINATION OF THE AIR SACS OF ORANGUTANS AND OTHER NONHUMAN PRIMATES
Examine the skin over the air sac for signs of fistulae or scars. Note thickness of the skin and presence of fat or muscle overlying the air sac.

Incise the air sac through the skin on the anterior aspect. Note color and texture of air sac lining. Note presence or absence of exudate.

Note presence or absence of compartmentalization by connective tissue and presence of diverticulae.

Note extent of air sacs (e.g., under clavicle, into axilla, etc.)

Identify and describe the opening(s) from the larynx into the air sac (e.g. single slit-like opening, paired oval openings etc.). Note any exudate.

Note the location, size and shape of the opening in the larynx (e.g. from lateral saccules or centrally at the base of the epiglottis). Note length of any connecting channel between larynx and air sac and direction a probe must take to go from inside the larynx to the air sac.
Cultures:

Please culture several different sites within the air sacs (we need data to determine normal flora and if infections are "homogeneous" or compartmentalized).

2/15/04; updated 8/13/201

Section 5.2.10. Avian Sampling Methods

Prepared by
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And the PREDICT One Health Consortium

Objectives: To safely collect biological samples from live and dead wild birds.

This document was made possible by the generous support of the American people through the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT program. It was drafted to support activities conducted under PREDICT and is intended for an audience of qualified professionals trained in standard, associated best practices. This guide is not intended for use by untrained individuals.

The authors assert that animal capture and sampling should always occur in compliance with all applicable laws and regulations and should only be undertaken after securing all necessary permits and approvals, including ethical approvals.

For more information about the contents of this guide, please contact predict@ucdavis.edu.

Suggested Citation Form: PREDICT One Health Consortium 2016. PREDICT Operating Procedures: Avian Sampling Methods.

Table of Contents: Avian Sampling

Section 5.2.10a. Confirmation of Knowledge

When you are familiar with the information in this guide, take the PREDICT quiz ***Section 8.4.9. Avian Sampling***.

Section 5.2.10b. Brief Overview of PPE

Minimum PPE Required for Handling Live, Dead, or Samples of Birds

The minimum PPE for bird sampling includes:

1. Designated clothing
2. Closed-toed shoes
3. Nitrile gloves
4. Protective glasses
5. N95 facemask for self-protection and to avoid contaminating samples.

(See the ***Biosafety and PPE Guide (Section 4.)*** for detailed instructions regarding PPE Use)

Section 5.2.10c. Avian Data Collection

Please refer to the following three templates for required data collection:

1. P2 Animal Data Collection Form
2. P2 Site Characterization Data Collection Form
3. P2 Specimen Data Collection Form

For more information on downloading templates from EIDITH see ***Section 5.2.3. General Data Collection Templates and Applications***.

Biometric Measurements

The P2 data templates mentioned above are required to be filled in. Additional biometric measurements may be collected at the discretion of the sampling party.

For many bird species, the sex or age of a captured individual may not be immediately evident with a simple visual inspection. Subtle but significant differences in morphology are often useful for differentiating between sexes and age classes.

Thus collecting biometric measurements can have important applications in disease sampling studies for determining differential infection or exposure rates based on sex or age.

Biometric measurements to be collected (optimal):

- Weight
- Culmen (bill) length and depth
- Tarsus length
- Wing length
- Tail length

Additional important data to establish breeding or physiological status of the bird:

- Presence of brood patches
- Moult stage

Age class:

- Usually the exact age will not be known and individuals should be assigned to a juvenile or adult age class

For collection methods for biometric measurements refer to FAO manual "Wild Birds and Avian Influenza: an introduction to applied field research and disease sampling techniques" (**http://www.fao.org/docrep/010/a1521e/a1521e00.htm**)

Photographs for Bird Identification
(Reference: European Commission DG Sanco 2006)

Digital photographs should be taken of each individual. The bird should fully fill the photographic frame, and wherever possible the image should include a ruler or other scale measure.

Photographs should be taken of:

- The whole bird, dorsal side, with one wing stretched out and tail spread and visible;
- The head in profile clearly showing the beak;
- Close-up photos of the tips of wing feathers can often determine whether the bird is an adult or a juvenile (bird in its first year);
- Ventral photographs should show the legs and feet (since leg color is often an important species diagnostic). If any rings (metal or plastic) are present on the legs, these should be photographed in situ as well as recording ring details.
- Any conspicuous markings/patterns should be photographed.

In late summer many water birds, especially ducks and geese, undergo moult and can be especially difficult to identify by non-specialists. At this time of year there is particular need for clear photographs to aid identification. The patch of color on the open wing's secondary feathers (called the "speculum") is often especially useful.

Section 5.2.10d. Avian Sample Collection

Samples are to be collected in duplicate from each animal. One sample must be collected into Trizol and one into viral transport media (VTM). Tubes must be labeled with a unique identifier number. Printed labels should be used (please see ***Section 5.2.3. General Data Collection Templates and Applications***).

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two oral swabs** - one in 500 µL VTM and one in 500 µL Trizol
2. **Two cloacal swabs** - one in 500 µL VTM and one in 500 µL Trizol
   and/or
   Two fecal samples - one with max of 500ul/0.5cc feces in 500 µL VTM and one with max of 500ul/0.5cc feces in 1 mL Trizol
3. **Two whole blood samples** - one with max of 500 µL of whole blood in 500 µL VTM and one with max of 500 µL of whole blood in 500 µL Trizol
4. **Two serum samples** - 2 x 0.5ml aliquots frozen without media

Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

If there is no **short-term** access (i.e., within 24 hours) to cold chain such as in an emergency situation, then samples can be collected in 500 µL of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

- 1 day at 37 °C (i.e., ambient temp)
- 1 week in the refrigerator
- Within one week freeze at -80°C for storage until analysis

<u>Details on Sample Collection and Storage Media</u>

1. **Two oral swabs**: Using sterile, polyester-tipped swabs with either an aluminum or plastic shaft, rub the swab tip gently but thoroughly against the back of the animal's throat, saturating the swab with saliva (see Figure 1).

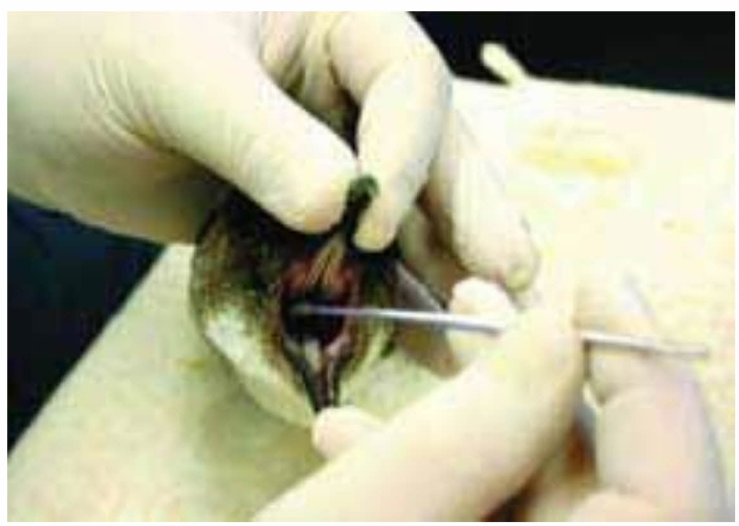

Figure Avian 1: Oral swab sample collection from a bird *(Photo credit: Taronga Zoo/Karrie Rose from FAO Wildlife bird highly pathogenic avian influenza surveillance manual)*

   Place 1 swab in a cryovial filled with 500 µL Trizol and use alcohol-wiped (or ethanol-wiped), flame-sterilized scissors to cut the shaft of the swab above the tip. [Note: If the plastic shaft can be snapped, then scissors are not necessary and the risk of cross-contamination is reduced. To snap the swab, lift the swab a little above the bottom of the vial then snap it. This will ensure the swab will not block the cap]. Place the other swab into 500 µL of VTM (= maximum final ratio of 1:1) in a cryovial.

Store in a liquid nitrogen dry shipper or dewar and transfer to -80˚C freezer when possible.

2. Two cloacal swabs/fecal samples: Gently and slowly insert the head of the swab into bird's cloacal cavity (cloacal cavities of small birds can be very shallow; thus the swab head should not be inserted very far into the cloaca). Swabs can be moistened with sterile saline prior to animal sampling. **Do not use VTM or Trizol to moisten swabs.** Gently twirl or rotate the swab back and forth 2-5 times to exfoliate (collect) cells from the cloacal wall (see Figure 2). Remove the swab from the cloacal cavity and place in a cryovial filled with 500 µL Trizol and use alcohol-wiped (or ethanol-wiped), flame-sterilized scissors to cut the shaft of the swab above the tip. [Note: If the plastic shaft can be snapped, then scissors are not necessary and the risk of cross-contamination is reduced. To snap the swab, lift the swab a little above the bottom of the vial then snap it. This will ensure the swab will not block the cap]. Repeat above process with second swab, and place into 500 µL of VTM (= maximum final ratio of 1:1) in a cryovial.

For fecal samples, add 500 µL or pea-sized piece of feces directly into 2 cryovials, one containing 1 mL Trizol (= maximum final ratio of 1:2) and one containing 500 µL VTM (= maximum final ratio of 1:1) and mix each tube well.

Store in a liquid nitrogen dry shipper or dewar and transfer to -80˚C freezer when possible.

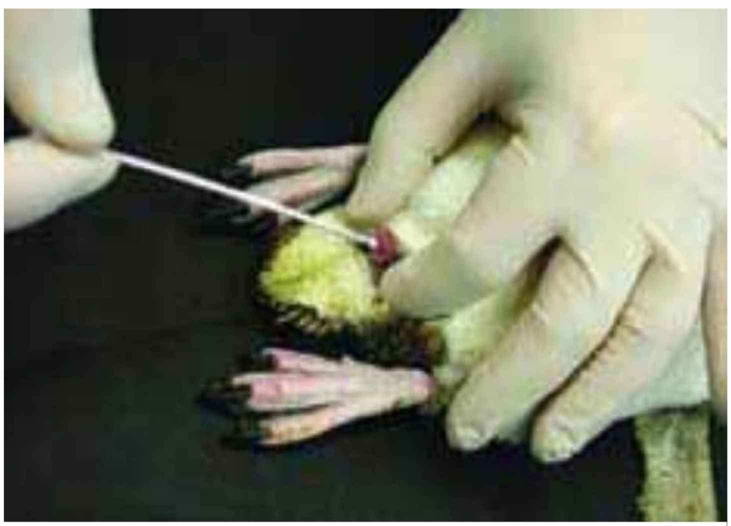

Figure Avian 2: Cloacal swab sample collection from a bird *(Photo credit: Taronga Zoo/Karrie Rose from FAO Wildlife bird highly pathogenic avian influenza surveillance manual)*

3. Whole blood and serum samples: Blood can be collected from the jugular vein (right side of the bird's neck; see Figure 3), brachial/ulnar vein (wing vein) or medial metatarsal vein (leg vein; see Figure 4) using a 22G, 23G, 25G, or 27G hypodermic needle hypodermic needs or butterfly needle and a 12 mL, 10 mL, 6 mL, 3 mL or 1 mL syringe, depending on the size of the bird and the amount of blood to be collected.

In general, it is safe to collect 0.3-0.6 cc of blood per 100 g of body mass from live birds, however, it is always best to collect as little blood as is necessary to conduct the testing required. If you plan to do hematology tests in addition to disease surveillance, it is recommended that you use a 22G through 25G needle as a 27G needle or smaller damages cells as they pass through this narrow diameter needle. After blood is collected, cover the venipuncture site with gauze and apply digital pressure until bleeding stops (30-60 seconds).

Add up to 500 µL of whole blood directly into 2 cryovials, one containing 500 µL Trizol and one containing 500 µL VTM (= maximum final ratio of 1:1) and mix each vial well.

Note: *If animals are too small to collect two blood tubes (for whole blood and serum), collect serum and save remaining clot in VTM after serum separation.*

For serum samples, collect blood using a non-heparinized syringe and place blood into a serum vacutainer (red-top) tube containing serum-clotting factor. After allowing the blood to clot, either spin tube in a centrifuge or allow tube to stand vertically on ice as much as possible. Use a sterile pipette tip and pipette gun to draw off serum and collect 2 x 0.5ml aliquots (no Trizol or VTM).

Store in a liquid nitrogen dry shipper or dewar and transfer to -80˚C freezer when possible.

Figure Avian 3: Blood sample collection from the jugular vein. *(Photo credit: FAO, 2007)*

Figure 4: Blood sampling from the medial metatarsal vein. *(Photo credit: FAO, 2007)*

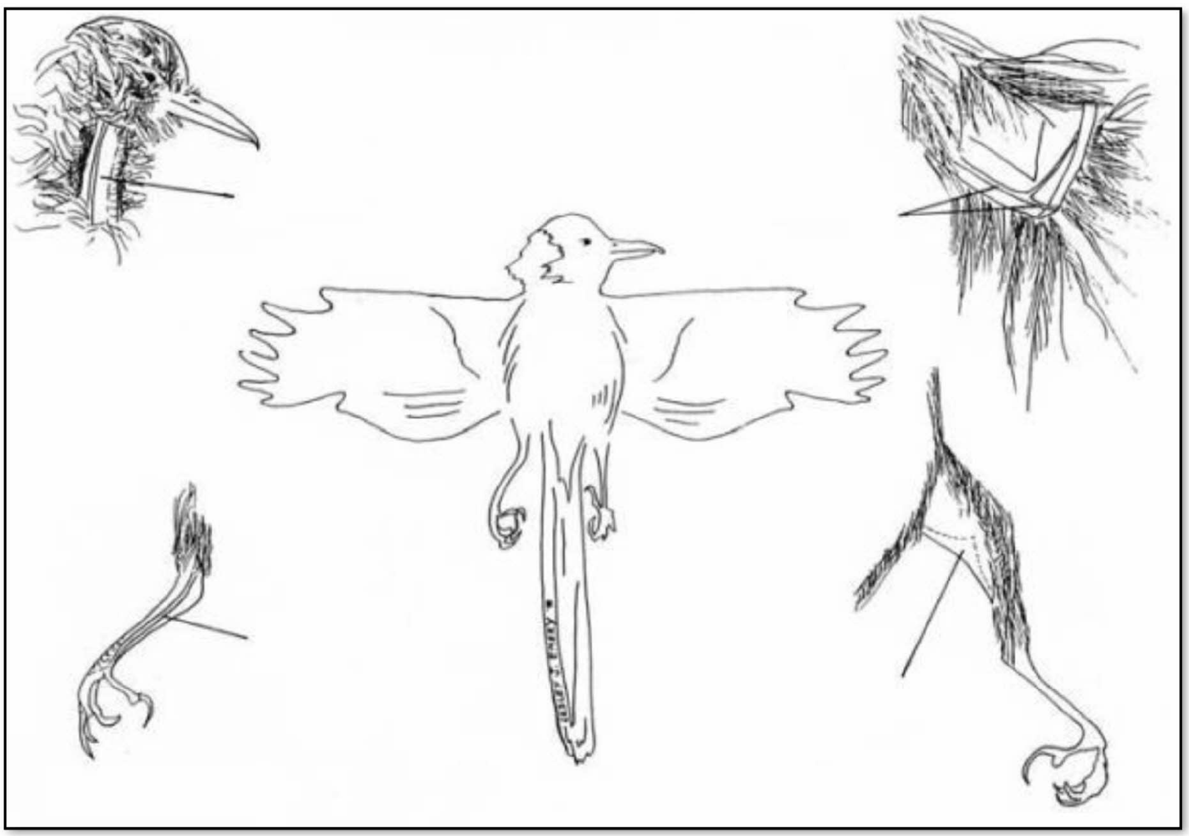

Figure Avian 5: Common sites of venipuncture and administration of subcutaneous fluids in birds *(Photo credit: FAO, 2006)*

Sampling of Dead or Euthanized Birds

If carcasses are not whole, the PREDICT guide for ***Bushmeat Sampling*** may be more applicable. If bodies are relatively whole and fairly fresh then blood, organ tissues and urine should be collected.

As discussed throughout this protocol, all wildlife should be considered potentially infectious for a wide variety of dangerous pathogens and dead animals in particular should be sampled only following all safety measures including proper use of PPE, proper work station decontamination and proper carcass disposal as outlined here and in other PREDICT documents (***Section 4. Biosafety and PPE Use***, ***Section 2.5. Safe Disposal of Carcasses and Infectious Waste Guide***, and ***Section 5.2.4. General Field Sampling Station Setup***).

Thorough necropsy procedures can be very beneficial and might pertain to some animals (e.g., valuable or known individuals, suspicious deaths, etc.); these procedures are addressed in separate documents. Time and skill permitting, when full necropsies are performed, following any Association of Zoos and Aquariums/AZA (or similar) necropsy protocol is recommended. Most of these protocols can be adjusted for application to other species. (Note: properly following extensive necropsy procedures and collecting and measuring all samples can require 4-6 hours for a single animal.)

Post-Mortem Blood Collection

In recently dead animals, it may be possible to collect whole blood (often clotted) from the right side of the heart, where the largest volume of blood is available. Blood may also be found in the chest cavity. If the animal died recently and the blood has not yet clotted, collect whole blood into 1 lavender top tube containing EDTA and add up to 500 µL of whole blood directly into 2 cryovials, one containing 500 µL Trizol and one containing 500 µL VTM. Also collect whole blood separately to obtain serum. Collect available blood into an appropriate sized container (typically one or more blood tubes) and allow it to sit undisturbed for at least 30 minutes. Then centrifuge at high speed (2000 x G for 20 minutes), remove the serum (clear, yellow or red-tinged fluid at the top), transfer clots into cryovials containing 500 µL Trizol and 500 µL VTM, and freeze the samples. If a centrifuge is not available, allow clots and cells to settle as much as possible and then collect serum into 2 x 0.5ml aliquots and blood clots into cryovials containing 500 µL Trizol and 500 µL VTM.

Tissue Collection from Dead Birds

Collect three, adjacent, approximately 200mg (pea-sized) samples of the following tissues:

- Adrenal
- Colon
- Heart
- Liver
- Lymph node
- Ovary
- Cecum
- Duodenum
- Kidney
- Lung
- Spleen
- Pancreas

- Testes
- Other, if required

One specimen should be frozen in 500 μL VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).

It will usually require experience to identify abnormal tissues, but potentially recognizable gross lesions include masses, discolored areas, ulcerations, etc. Samples for histopathology (i.e., in formalin) should be collected at the abnormal margins to include both normal and abnormal sections in the same piece of tissue. Collection of any obvious internal parasites in ethanol is also recommended.

Section 5.2.10e. Health and Welfare of Birds during Capture and Handling

The health and well-being of the birds is the primary concern during all phases of capture. There are multiple methods for trapping and handling varying bird types, and examples can be found in the FAO Animal Production and Health Manual No. 5 ("Wild Bird HPAI Surveillance: sample collection from healthy, sick and dead birds", available at: http://www.fao.org/avianflu/en/animalhealthdocs.html)

The following principles should be adhered to, to ensure birds are captured and handled correctly, safely and with minimum disturbance (FAO, 2007):

- Wild bird capture is strictly controlled in most countries; those engaged in capture activities should be aware of and comply with local and national laws and obtain all required local, state, provincial & federal permits well in advance.
- Capture techniques and equipment that expose birds to foreseeable risk of injury should be avoided at all costs.
- Approved restraint techniques and handling guidelines should be used e.g., those described by FAO (2007); consult with experienced wildlife veterinarians and biologists if modifications to restraining and handling techniques are required.
- Those conducting capture efforts should take all precautions to avoid disturbing nesting birds at breeding sites or enhancing vulnerability to nest site predation following human intrusion.
- Monitor weather forecasts prior to conducting capture efforts to ensure birds are not captured during extreme climatic conditions that would expose them to an increased risk of hypothermia or hyperthermia.
- Always have a sufficient number of experienced personnel (at least four) available before undertaking any capture operation.
- Check operative traps and nets at appropriate time intervals; birds should not remain in traps or nets any longer than is necessary. This is capture technique and weather dependent, and could be as short as every 15 minutes to twice a day.
- Close or dismantle traps and nets that are inoperative and not checked regularly.

- Maintain a calm and quiet environment at the bird-handling site.
- Conditions at the bird-processing site should be appropriate for the environmental conditions: in cold, wet conditions, birds should be kept warm and dry; in hot, sunny conditions, birds should be processed in a sheltered, shaded and cool site.
- Processing stations should be located as near as possible to the capture site to avoid holding birds for transportation any longer than is absolutely necessary.

Bird Welfare (FAO, 2007)
There is always the risk of distress or injury when handling wild birds. Preferably, an appropriately trained veterinarian will be available to examine and treat any injured or distressed bird, but, at the very minimum, a basic first aid kit should be included in the equipment list of every field study. In no instance should a seriously injured bird be released into the wild without first being examined and treated by a veterinarian. If euthanasia is required see the AAZV and ***AVMA guidelines (Section 8.5.2)***.

Common Maladies and Treatments

Scratches, cuts, and abrasions
These may be unavoidable during capture and confinement and simple treatment by rinsing the injury with clean water or sterile saline before releasing the bird should suffice for most minor injuries. More serious injuries should be brought to the attention of a veterinarian.

Shock/Inertia
Birds are susceptible to the stress of capture and handling and may suffer a physiological (shock) or neurological (inertia) reaction where birds become unresponsive to external stimuli to the point that they appear "frozen". Shock may be accompanied by rapid breathing (not evident in inertia).

Birds should be allowed to recover in a quiet, sheltered and well-ventilated area, well away from any human activity. Limiting time in captivity, maintaining a calm and quiet captive environment, and working at a site appropriate for the environmental conditions will help prevent shock and inertia.

Hypothermia and Hyperthermia
Capturing, transporting and handling birds during extreme temperatures, rain or foul weather makes them vulnerable to hypothermia or heat stress (hyperthermia) and should be avoided where possible.

Hypothermia can occur in cold conditions when feathers become wet and lose their insulating properties. Signs of hypothermia include shivering, lethargy and skin that is cold to the touch. Birds suffering from hypothermia should be dried and placed near a heat source such as a heating lamp (compact fluorescents bulbs should be at least 4-6" (10-15.25cm) from the animal's head and UV bulbs 12-20" (30.5-50.8cm)) or a hot water bottle (non-insulated). Holding wet birds in dry airy crates, at sufficiently low density and away from human disturbance usually allows them to preen themselves dry.

Handlers should avoid use of petroleum-based lotions (e.g., common in hand-creams and moisturizers) that may cause plumage to lose its insulating properties.

Hyperthermia can occur in hot conditions when birds are held in direct sunlight, at high ambient temperatures, or in overcrowded crates without adequate ventilation or water. Hyperthermia may also occur if birds are subject to a prolonged chase during capture. Signs of hyperthermia include panting, wings held away from the body, lethargy, seizures or prostration.

Birds suffering from hyperthermia should not be handled, but should be placed in a well-ventilated box/crate, moved to a cool, shaded area and provided with abundant drinking and swimming water. It may be beneficial to mist the bird with water or apply alcohol or water to the bird's feet to accelerate heat dissipation.

Section 5.2.10f. References

European Commission, DG SANCO (2007).
EU Guidelines for AI surveillance in wild birds and poultry in 2007.

FAO 2006. Wild Bird Highly Pathogenic Avian Influenza Surveillance: Sample collection from healthy, sick and dead birds. Edited by K. Rose, S.H. Newman, M. Uhart and J. Lubroth. FAO Animal Production and Health Manual, No. 4. Rome

FAO. 2007. Wild Birds and Avian Influenza: an introduction to applied field research and disease sampling techniques. Edited by D. Whitworth, S.H. Newman, T. Mundkur and P. Harris. FAO Animal Production and Health Manual, No. 5. Rome. (also available at www.fao.org/avianflu)

Section 5.2.8. Rodent Sampling Methods

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Objectives: To safely collect biological samples from live and dead rodents.

This document was made possible by the generous support of the American people through the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT program. It was drafted to support activities conducted under PREDICT and is intended for an audience of qualified professionals trained in standard, associated best practices. This guide is not intended for use by untrained individuals.

The authors assert that animal capture and sampling should always occur in compliance with all applicable laws and regulations and should only be undertaken after securing all necessary permits and approvals, including ethical approvals.

For more information about the contents of this guide, please contact predict@ucdavis.edu.

Suggested Citation Form: PREDICT One Health Consortium 2016. PREDICT Operating Procedures: Rodent Sampling Methods.

Table of Contents: Rodent Sampling Methods

Section 5.2.8a. Confirmation of Knowledge

When you are familiar with the information in this Guide, take the PREDICT quiz ***Section 8.4.7. Rodent Sampling.***

Section 5.2.8b. Brief Overview of PPE

Minimum PPE Required for Handling Rodents

The minimum PPE for handling of rodents during capture and sampling includes:

1. Designated long-sleeved clothing
2. Nitrile extended cuff gloves[1]
3. Eye protection
4. N95 facemask
5. Washable shoes

(See the ***Biosafety and PPE Guide (Section 4.)*** for detailed instructions regarding PPE Use)

Section 5.2.8c. Data Collection

Please refer to the **required data collection templates** for data to collect:

1. P2 Animal Data Collection Form
2. P2 Site Characterization Data Collection Form
3. P2 Specimen Data Collection Form

For more information on downloading templates from EIDITH see ***Section 5.2.3. General Data Collection Templates and Applications.***

Section 5.2.8d. Rodent Capture, Handling, and Sampling

Capture techniques will vary based on the species being targeted and the location where the samples are being collected. Details of the main techniques, including information on Sherman traps, are available in other documents such as the CDC guide *Methods for Trapping and Sampling Small Mammals for Virologic Testing* (pp 15-18) (**http://www.cdc.gov/hantavirus/pdf/rodent_manual.pdf**).

Note*: Field staff must use this PREDICT guide for specimen collection techniques.*

[1] Nitrile gloves are recommended for handling rodents, in the absence of nitrile gloves and allergies to latex, double latex gloves could be considered.

The PPE requirements for handling animals during capture or during processing are the same. All animal capture, handling, and sampling must also be done in accordance with your current in-country IACUC approval, which should be amended accordingly and be consistent across the pertinent approvals.

Rodent Capture and Handling

***Note:* Country Coordinators are responsible for following all local laws and regulations regarding veterinary anesthetic drug use.**

1. Free-ranging rodents will be captured using metal box traps (Sherman/Tomahawk traps). Traps will be set open at sunset and checked at sunrise. If adverse weather is expected, such as abrupt changes in precipitation intensity that could cause flash flooding events/animals getting wet, or if researchers are working in areas where predation is common, traps will be checked more frequently, and those traps will be closed during the adverse weather event. Traps will be located in areas protected from direct sunshine (e.g. ground cover vegetation or artificial reflective foil insulation) to prevent consequent heat stress. Otherwise, traps will be closed between sunrise and sunset.

2. To avoid hypothermia, allow thermoregulation, and reduce capture stress, always provide nesting material (e.g., pulped cotton fiber, such as Nestlets by Ancare) to each trap. If the animal is in hypothermic shock, wrap the animal in a dry drape, place a hand warmer (e.g., reusable exothermic pad or air-activated disposable pad) in the outer layer of the wrap, place the animal in a dry recovery chamber (i.e. clear plastic box), monitor recovery and then release at the capture location.

3. Remove animals from traps by placing a clear plastic handling bag or large Ziploc-style bag over the trap, opening the door into the bag and coaxing animal out by gently shaking the trap. DO NOT BLOW INTO THE TRAP.

4. Weigh the animal in the handling bag using a Pesola scale or a flat digital scale prior to inserting the anesthetic ball (see below). Temporarily record the animal plus bag weight and after the animal is anesthetized and removed, reweigh the empty bag and subtract the bag weight from the total to obtain the animal weight.

5. Scruffing is the best way to handle smaller rodents for sampling. Hold the anesthetized animal by pinching the skin between the thumb and forefinger at the point where the rodent's spine meets the head (Figure 2). For a more secure hold and/or access to the ventral surface, position the rodent's body firmly across your hand by extending your forefinger and thumb back as far as possible while maintaining a firm grip on the scruff. Place the tail between the fingers of this same hand (Figure 3).

Note: If you grasp too much skin, the airway will become restricted and the rodent will become cyanotic. Monitor the condition of the animal the entire time it is restrained, being careful to observe the breathing rate and color of the ears, nose, and oral cavity. The animal should be released immediately if there are any signs of gasping or change in coloring from pink to blue.

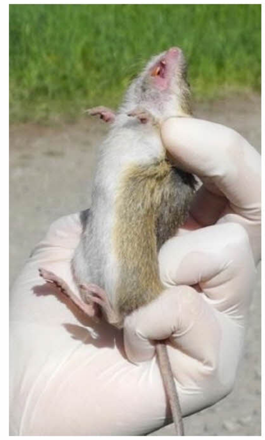


Figure 1 (left): scruff restraint technique. Figure 2 (right): positioning the animal in the hand while scruffing, in order to collect samples. Both: © Leticia Gutiérrez Jiménez.

Rodent Anesthesia

Anesthetize rodents with isoflurane.
***Note**: Pregnant women should avoid or minimize exposure to isoflurane.*

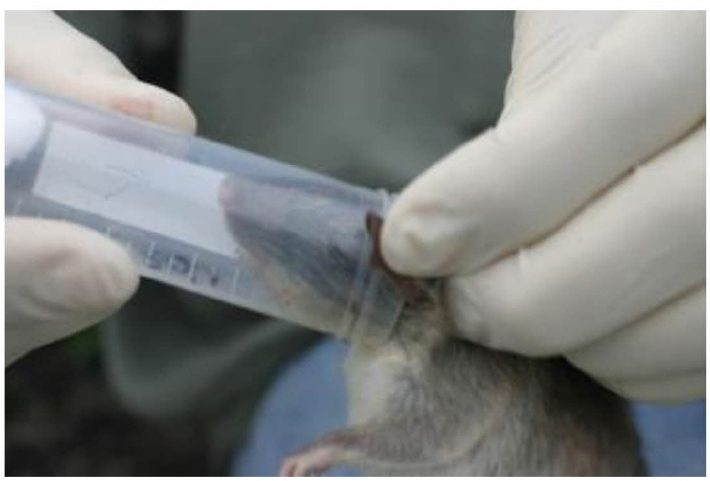


Figure 3: Nose cone for anesthesia. Figure: PREDICT

1. Keep in mind species-specific Isoflurane hypersensitivity (e.g. voles) and that age, sex, and reproductive stage can affect drug potency, which in turn may cause abrupt respiratory depression - especially in older individuals.

2. Apply 0.4 ml of isoflurane to a cotton ball and put the cotton ball into a metal tea ball or plastic tube (nose cone; 0.4 ml is the appropriate dose for a ca. 20g mouse, adjust the dose as needed for larger rodents). If tea ball is used, place the metal ball into the clear plastic holding bag or container with the rodent; if nose cone is used, place the rodent's nose into the tube.

3. Watch the animal closely until anesthetized--Breathing rate will increase then slow as the animal progresses further under anesthesia.

4. When withdrawal reflex is suppressed (use toe pinch to measure the withdrawal reflex), remove rodent from bag for processing.

5. Place an additional cotton ball soaked with 0.2 ml isoflurane into a 50 ml tube and screw on the cap. If the animal begins to wake up during blood collection, unscrew the cap of the nose cone and position over the animal's nose for as brief a time as possible to re-anesthetize the animal (see Figure 1 below). A nose cone for supplementary anesthesia should be used with care as different animals respond differently to isoflurane inhalation.

6. Collect samples immediately after the rodent is anesthetized.

Note: For larger rodents, chemical restraint and anesthesia (ketamine alone 25-50mg/kg, or ketamine combined with xylazine 80-100 mg/kg ketamine + 10-12.5 mg/kg xylazine) can be applied. Drugs can be administered to manually-restrained or squeeze-caged animals by syringe or by blow dart, depending on size and situation.

Basic Sample Set for Rodent Sampling

The following basic set of samples should be collected from each animal where possible. *If only one sample can be collected, then place into VTM.*

1. **Two oral swabs** - one in 500 µL VTM and one in 500 µL Trizol
2. **Two rectal swabs** - one in 500 µL VTM and one in 500 µL Trizol
   or
   Two fecal samples - one with max of 500 µL/0.5cc feces in 500 µL VTM and one with max of 500 µL/0.5cc feces in 1 mL Trizol
3. **Two urogenital swabs/urine samples** – one in 500 µL VTM and one in 500 µL Trizol
4. **Two whole blood samples** - one with max of 500 µL of whole blood in 500 µL VTM and one with max of 500 µL of whole blood in 500 µL Trizol
   Note: *If animals are too small to collect two blood tubes (for whole blood and plasma/serum), collect plasma/serum and save remaining clot or red cell fraction in VTM after plasma/serum separation.*
5. **Two serum samples** - 2 x 0.5ml aliquots frozen without media

Swab Sample Collection

1. Collect **2 oral swab** samples using sterile, polyester-tipped swabs with plastic or aluminum shafts. A sterilized handle of a pair of forceps can be used to safely open the animal's mouth. Rub the polyester tip of the swab gently, but thoroughly, against the back of the animal's throat. Place one swab into a cryovial containing 500 µL of VTM and one in a cryovial with 500 µL of Trizol. Cut the shaft of the swab with alcohol-wiped (or ethanol-wiped), flame-sterilized scissors. To cut the swab shaft, lift the swab a little above the bottom of the vial and then cut it. If the shaft is cut when the swab tip is resting on the bottom, the swab will be too long and the cryovial won't close.

2. Collect **2 rectal swabs** using appropriately-sized, sterile polyester swabs. For smaller animals, use urethral or pediatric swabs. Rectal swabs can be moistened with sterile saline prior to animal sampling.

   DO NOT USE VTM TO MOISTEN SWABS BEFORE SAMPLING
   DO NOT USE TRIZOL AS A LUBRICANT *– IT IS HIGHLY IRRITATING TO TISSUE.*
   DO NOT FORCE TIP OF SWAB INTO RECTUM*. IF IT WON'T ENTER EASILY, DO NOT COLLECT THIS SAMPLE.*

   Gently insert one sterile swab tip at a time into the animal's rectum. Place 1 swab in a cryovial filled with 500 µL of VTM, and using isopropyl alcohol-wiped (or ethanol-wiped), flame-sterilized scissors, cut the shaft of the swab. Place the other swab into a cryovial with 500 µL of Trizol. Store in a dewar or dry shipper with liquid nitrogen and transfer to -80˚C freezer when possible.

   Alternatively, collect fresh feces: Add 500 µL (pea-sized) pieces of feces directly into two vials, one containing 500 µL VTM (maximum final ratio of 1:1) and one containing 1 mL Trizol (maximum final ratio of 1:2) and mix each tube well. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80˚C freezer when possible.

3. Collect **2 urogenital swabs** and place one in 500 µL of VTM and one in 500 µL of Trizol. Swabs can be moistened with sterile saline prior to animal sampling. If the animal urinates, collect up to 500 µL of **urine** using pipettor and place into one cryotube with 500 µL of VTM and mix well. Pipette up to another 500 µL of urine into a cryotube with 500 µL of Trizol and mix well.

Blood Sample Collection

Make sure the total blood collected from the animal does not exceed 1% of weight of animal (i.e., a maximum of 500 µL should be collected from a 50g rodent).

If animal size allows, collect blood and place in a red-top vacutainer tube with clotting factor and in a lavender-top tube (e.g., EDTA). Allow blood in red-top tube to clot, then centrifuge and pipette two 500 µL serum samples and freeze without media. From lavender top tubes, add up to 500 µL of whole blood to a cryotube with 500 µL of VTM and 500 µL of blood to a second cryotube with 500 µL of Trizol (if blood volume is <500 µL then only collect into VTM).

If animals are too small to collect vacutainer blood tubes, blood collection can be done with Sarstedt Inc Microvette Capillary Blood Collection Tubes (100ul to 600ul capacity), Becton Dickenson BD Microtainer™ Capillary Blood Collector and BD Microgard™ Closure tubes (250ul-500ul capacity), Terumo™ Capiject™ capillary blood collection tubes (500ul capacity), or heparinized 75 µL glass hematocrit tubes.

For blood collected into hematocrit tubes, use a bulb to expel the whole blood in a cryotube with 500 µL of VTM. Hematocrit tubes can be centrifuged using portable hematocrit or standard vacutainer centrifuges to separate plasma. Score glass tube using a razor blade or X-acto knife where the serum meets the red cell fraction and snap the tube. Use a bulb to expel plasma into a micro-cryovial and freeze. If two or more capillary tubes are filled, collect two aliquots of plasma. Preserve the remaining red cell fractions in a separate cryovial with VTM and freeze.

Guidelines for Selecting Bleeding Method

Rodent Taxa	Mice, field mice, jumping mice	Voles, gerbils, and hamsters	Squirrels and chipmunks	Rats and rat-like species
Bleeding Method	*1) Facial vein* *2) Saphenous vein*	*1) Facial vein* *2) Saphenous vein*	*1) Saphenous vein*	*1) Saphenous vein* *2) Lateral tail vein* ***Larger animals:*** *Ventral tail vein, jugular vein*

Table 1: Recommended Bleeding Technique(s) by Rodent Taxa

Local personnel should be acquainted with the local fauna and their anatomy and apply this knowledge to determine the most appropriate sampling methods to use in the field. Do not attempt venipuncture without at least one field person present with prior experience, especially with the facial vein and jugular vein bleeding approaches.

General Techniques for Bleeding Rodents

NOTE: Bleeding by any method should only be performed on anesthetized animals.

SUBMANDIBULAR OR FACIAL VEIN BLOOD COLLECTION

Adapted from the MEDIpoint, International Inc. Goldenrod lancet 'For use on mice' website.

For videos of the technique, please visit
http://www.medipoint.com/html/pc_video_instructions.html (for PC)
http://www.medipoint.com/html/mac_video_instructions.html (for Mac)

For this technique you will need to purchase lancets to prick the skin on the rodent's cheeks.[2] You can use any lancet, but as guidance, Goldenrod lancets are recommended. They come in four point lengths: 3, 4, 5 and 5.5mm point. You should use the best option depending on the age/size of the rodents you will sample. If you only need one or two drops, use 3mm point (keep in mind that a standard blood drop is equal to ~50ul). Always be careful that the volume of blood harvested does not exceed the total volume recommended for that size animal's safety and health (≤1% body weight).

Step-by-step procedure:

1. Choose the proper lancet point length (see above), corresponding to the size of rodent.

2. Open the lancet/needle wrapper and prepare a collection vial.

3. Hold the anesthetized animal by pinching the skin at the back of the head (scruff restraint technique – see Figure 2) and taking the tail between the fingers of the same hand (Figure 3).

4. Locate the back of the jaw bone, the submandibular area. There is a vascular bundle located at the rear of the jaw bone providing a convenient and consistent source of blood (Figures 4, 5, and 6).

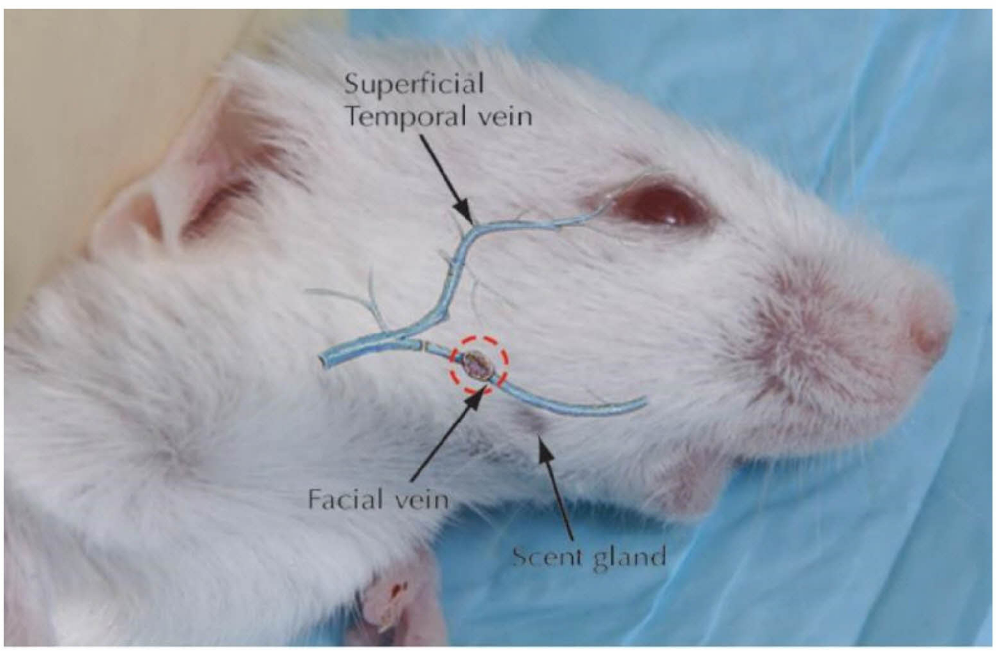
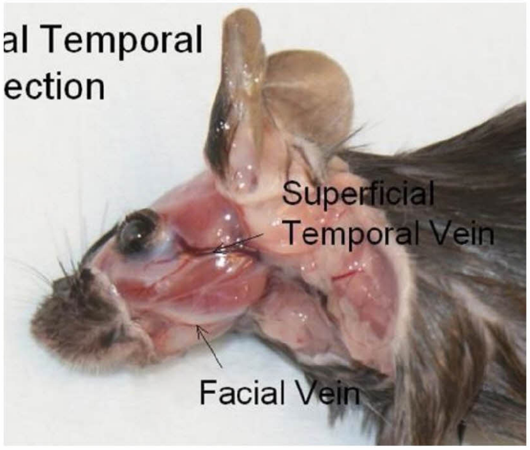


Figure 4: The vascular bundle targeted during blood collection from the facial vein. The dashed circle indicates the puncture site. Figure: Golde, Gollobin, and Rodriguez, 2005.

[2] Lancets provide the greatest control of puncture depth and are the recommended device for facial bleeding; however, sterile 20G-22G hypodermic needles may also be used **after** the facial bleeding technique has been completely mastered.



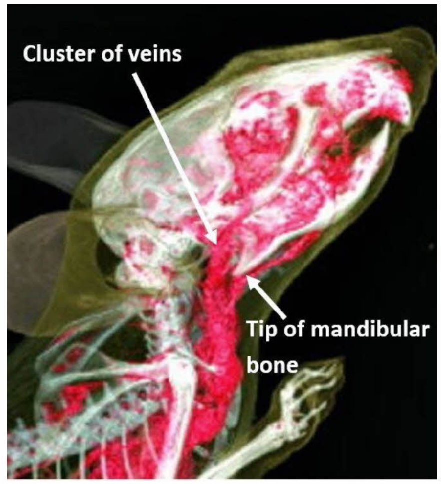


Figure 5 (left): Superficial temporal vein and facial vein in the mouse. Figure 6 (right): The targeted cluster of veins is just behind and above the tip of the mandibular bone. Both: MEDIpoint International, Inc.

5. Once the site is located, properly align the tip of the lancet: The facial muscles of a mouse or rat run fairly parallel to the bottom of the jaw line, that is, along the face from the nose/whiskers towards the ear. Align the lancet with the striations of the muscles in the animal's face so that when the tip enters the facial muscles, it will go between the striations instead of across them. This will cause less damage to the muscle tissue and therefore cause less scarring, and there will be less chance of hematoma formation.

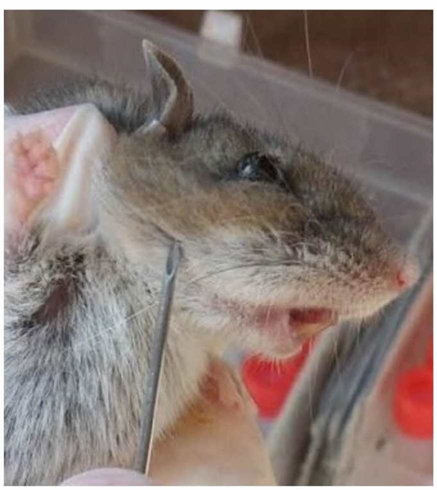



Figure 7 (left): Facial vein puncture site in P. maniculatus. Image: © Leticia Gutiérrez Jiménez. Figure 8 (right): After locating the puncture site, apply enough pressure to insert only the tip of the lancet into the cheek.

6. Quickly poke the cheek of the animal, applying enough pressure to insert only the tip the lancet, and withdraw immediately. A blood drop will form instantly. Approach the tube to collect the blood as it flows freely (Figures 7 and 8).

Note: To avoid sample contamination, do not rub or press the collection tube against the puncture site.

Figure 9: Collect blood into the vial as it flows freely, avoiding rubbing or pressing the tube against the puncture site. Both: MEDIpoint International, Inc.

If you are not getting enough blood, try the following technique: When scruffing the animal, hold the skin on the back of its neck between your thumb and middle finger. When more blood is desired, put your index finger on the top of the rodent's head and gently move its head up and down. This will keep the wound open a little longer and pump more blood into the submandibular area and out the puncture site.

Blood may also be drawn from both cheeks at one sampling event if the amount of blood to be collected is large.

7. Stop the blood flow at any time by releasing the head/neck scruffing pressure point and applying a compress (gauze, cotton) to the puncture site for a few seconds (or 1-3 minutes to prevent hematoma formation), as needed.

Note: Always ensure complete hemostasis before returning the animal to its individual trap.

8. To avoid hypovolemic shock in small animals (≤200g), fluid volume replacement should be administered subcutaneously after collecting all biological samples and immediately before placing the individual into its trap/recovery chamber. (See Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines)

Note: Recumbent or dehydrated individuals must be treated immediately and should **NOT be sampled**. Those animals should be released at the trap capture location as soon as they recover. To avoid dehydration and hypoglycemia, always provide a small apple or other fruit slice (1 apple = ~16 slices) as part of the bait mix and to each trapped individual while it is waiting to be processed.

LATERAL SAPHENOUS VEIN BLOOD COLLECTION

Adapted from Guide to the Care and Use of Experimental Animals. Volume 1, Second Edition. University of British Columbia.

For videos of this technique, please visit
https://norecopa.no/films-and-slide-shows/mouse
Blood sampling of the rat from the saphenous vein

A minimum of two people are required for this type of blood collection in rodents: one for anesthetizing, handling and bleeding, and one for collecting the blood sample and data recording.

A volume of 100µ -300 µl can readily be collected using the lateral saphenous vein bleeding technique; however, the number of attempts to take a blood sample should be minimized. Staff should make no more than three needle sticks in any one attempt. Warming the animal immediately prior to blood collection will increase blood flow considerably.

Step-by-step procedure:

1. Choose the appropriate anesthesia protocol and secondary restraining technique for the species. Mechanic and manual restraint causes stress, and therefore the duration of restraint should be minimized. Where a restraint tube is used, it should be appropriate to the size of the rodent. All forms of restraining equipment should be frequently washed to prevent pheromonally-induced stress or cross-infection.

2. The animal is held head-first in a restrainer so that only the rear legs and tail are free. The rear leg can be stretched out to its natural position. The head, nose, and ear color should be monitored closely. Apply the isoflurane nose cone for a couple of seconds if needed. For larger – and feisty – rodents, it is recommendable to wrap the animal in a drape or towel in case of premature awakening.

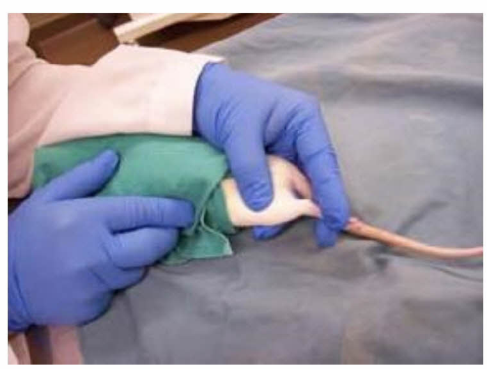


Figure 10 (left): Mouse or vole restraint. Figure 11 (right): Rat or squirrel restraint. Both: Guide to the Care and Use of Experimental Animals.

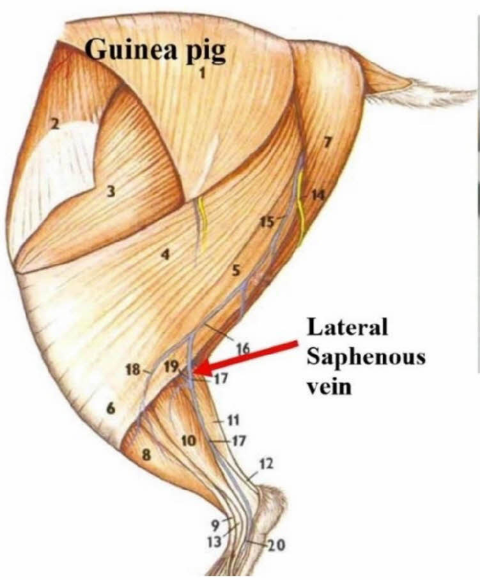
3. To secure the animal and elevate the vein, the skin on the upper thigh is gently but firmly squeezed, using the same hand holding the restrainer.

4. The vein is easier to see when the fur is shaved either by clippers or by using scissors. A thin film of bland ointment, such as petroleum jelly or Glycerin, can be applied to prevent blood from seeping and allow for blood drop formation. If you are not shaving the area, find the vein by “combing” or clearing away the fur using a cotton-tipped swab t has been dipped in jelly.



Figures 12-14 (left to right): Clearing the fur on the mouse, the rat or squirrel and the vole. Figures 12 and 13: Guide to the Care and Use of Experimental Animals. Figure 14: © Leticia Gutiérrez Jiménez

5. Locate the vein.



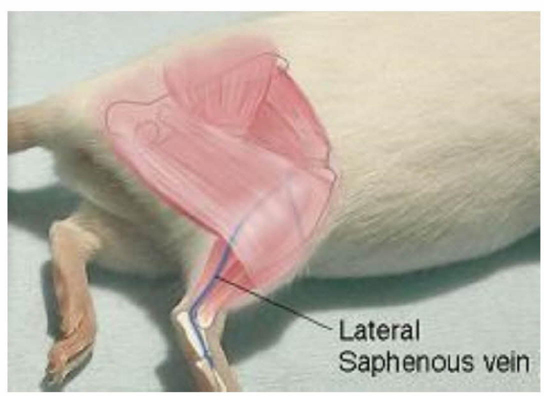


Figure 15 (left): The lateral saphenous vein in the guinea pig. Figure: Guide to the Care and Use of Experimental Animals. Figure 16 (right): The lateral saphenous vein in the mouse. Figure: Theodora.com

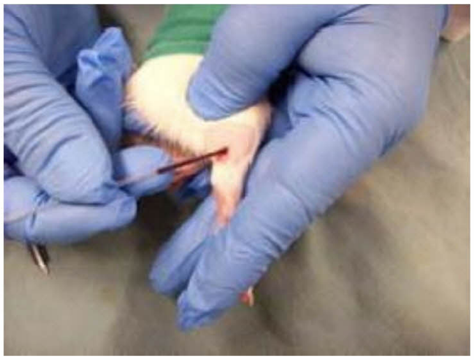
Figure 17: Lateral saphenous vein in the Least chipmunk *(Tamias minimus).* Figure: © Leticia Gutiérrez Jiménez

6. Using a **23-27 gauge needle or an appropriate depth size animal lancet**, puncture the vessel at a 90º angle at the most proximal visible aspect. Collect blood as it drips from the vein.

Figures 18-20 (left to right): Puncturing the lateral saphenous vein of the mouse, rat or squirrel, and vole. Figures 18 and 19: Guide to the Care and Use of Experimental Animals. Figure 20: © Leticia Gutiérrez Jiménez

Rodent taxa	Mouse (25g)	Hamster/Vole	Rat (250g)
Max. sample volume	200ul	0.5% bodyweight	1600ul
Needle size	27G-25G needle or lancet	25G needle or lancet	23G needle or lancet

7. Collect your sample.




Figures 21(left) and 22 (right): Collecting blood from the rat/squirrel and vole via the lateral saphenous vein. Figure 21: Guide to the Care and Use of Experimental Animals. Figure 22: © Leticia Gutiérrez Jiménez

8. Use a dry gauze pad and/or povidone-iodine prep pad to apply pressure to the puncture site as the pressure on the upper thigh is released. If bleeding does not stop, use a silver nitrate styptic pencil (moisten the pen slightly with sterile saline solution and then carefully place onto the puncture site). Silver nitrate will stop vigorous hemorrhage, but it stings sharply on the application site and should not be used habitually to stop minor bleeding.

9. In small animals (≤200g), fluid volume replacement should be administered subcutaneously after collecting all biological samples, as described in Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines.

<u>Note:</u> Recumbent or dehydrated individuals must be treated immediately and should **<u>NOT be sampled</u>**. Those animals should be released at the trap capture location as soon as they recover. To avoid dehydration and hypoglycemia, always provide a small apple or other fruit slice (1 apple = ~16 slices) as part of the bait mix <u>and</u> to each trapped individual while it is waiting to be processed.

10. Remove the rodent from the restrainer and place it in its individual trap.

11. Monitor the animal for 5-10 min to ensure hemostasis (bleeding has stopped).

<u>Techniques for Bleeding Rats and Larger Rodents</u>

LATERAL TAIL VEIN BLOOD COLLECTION

1. Use a tourniquet. The veins are located on either side of the tail and are quite superficial.

2. Use a needle with the appropriate gauge (usually 27G or 25G), entering the skin at a shallow angle about one third down the length of the tail. Be careful not to collapse the vein.
3. Consider using a small syringe or hematocrit tubes to collect the blood.
4. In small animals (≤ 200g) administer fluids after sampling as described above and in *Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines*.
5. Remove the rodent from the restrainer and place it in its individual trap.
6. Monitor the animal for 5-10 min to ensure hemostasis (bleeding has stopped).

VENTRAL TAIL VEIN BLOOD COLLECTION

1. Use a tourniquet, as above (lateral tail vein bleeding).
2. The vein is located both centrally and ventrally in the tail. The ventral tail vein is deeper than the lateral tail vein used in mice and it is not readily visualized. Take care not to bleed from the ventral caudal artery, which is sheathed by a thick fascia.
3. As with lateral tail vein bleeding, start one third of the way down the tail.
4. After bleeding, ensure hemostasis by applying pressure to site of bleeding using a cotton ball or gauze until bleeding ceases (approximately 1 minute).
5. Administer fluids as described above and in *Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines*.
6. Monitor the animal for recovery.

JUGULAR VEIN BLOOD COLLECTION

Jugular vein bleeding is recommended only for larger rodents (e.g., guinea pigs, chinchillas, etc.), and even then should be considered only if appropriate alternatives are impossible or have been unsuccessful. The technique requires a potentially challenging degree of animal restraint and a strenuous physical positioning of the animal to be sampled, which can be difficult with alert and resistant animals. A high level of competence is required in the animal sampling staff to avoid permanent harm to the animal.

1. On the anesthetized animal, clean and apply pressure to the jugular vein on one side.
2. Direct the needle into the vein and collect the sample.
3. After bleeding, ensure hemostasis by applying pressure to site of bleeding using a cotton ball or gauze until bleeding ceases (approximately 1 minute).
4. In the case of small animals or visibly dehydrated animals, administer fluids as described above and in *Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines*.
5. Monitor the animal for recovery.

Necropsy Sample Collections

In case of accidental death before or during animal sampling, or where dead animals are available opportunistically for sampling, perform a necropsy examination and collect the samples described above. If euthanasia is required see the AAZV and ***AVMA guidelines (Section 8.5.2)***.

In all cases, samples going into VTM and Trizol should be collected sterilely, limiting cross-contamination between tissues (see below). Collect as much blood as possible via cardiac puncture. In addition, collect **three** adjacent, approximately 200 mg (pea-sized) samples of the following tissues:

- Adrenal
- Cecum
- Colon
- Duodenum
- Heart
- Kidney
- Liver
- Lung
- Lymph node
- Spleen
- Ovary
- Pancreas
- Testes
- Other, if required

One specimen should be frozen in 500 μL VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).

To avoid cross contamination among individual specimens, follow sterile tissue collection technique.

1. Use clean scalpel handle, scissors and tweezers (Clean instrument = wiped with 70% EtOH and flamed). Use a <u>new,</u> sterile scalpel blade to cut skin and to open body cavities. Discard the used scalpel blade.
2. Open the abdominal and thoracic cavities carefully to prevent contamination between cavities and among organs.
3. Observe organs, noting abnormalities.
4. Use a new scalpel blade for specimen collection. Secure aseptic specimen collection by wiping (EtOH 70%) and flaming the instruments between every tissue sample.

Section 5.2.8e. References

Francisco, C.C., G.S. Howarth, and A.L. Whittaker. (January 2015) Effects on Animal Wellbeing and Sample Quality of 2 Techniques for Collecting Blood from the Facial Vein of Mice. Journal of the American Association for Laboratory Animal Science. 54(1): 80-84.

Hudson, P. and J. Sinclair. (2010) Mouse Research Standard Operating Procedures. Pennsylvania State University.

Lee, G. and K.A. Goosens. (May 2015) Sampling Blood form the Lateral Tail Vein of the Rat. Journal of Visualized Experiments. (99) e52766, doi:10.3791/52766 https://www.jove.com/pdf/52766/jove-protocol-52766-sampling-blood-from-the-lateral-tail-vein-of-the-rat

MEDIpoint International, Inc. Goldenrod Animal Lancet: For Use on Mice. http://www.medipoint.com/html/for_use_on_mice.html

Mills, J.N., J.E. Childs, T.G. Ksiazek, C.J. Peters, and W.M. Velleca. (1995) Methods for Trapping and Sampling Small Mammals for Virologic Testing. U.S. Department Of Health & Human Services, Centers for Disease Control and Prevention. 61pp.

Olfert, E.D., B.M. Cross, and A.A. McWilliam, eds. (1993) Guide to the Care and Use of Experimental Animals. Volume 1, Second Edition. University of British Columbia. https://www.ccac.ca/Documents/Standards/Guidelines/Experimental_Animals_Vol1.pdf

Quesenberry and Carpenter. (2004) Ferrets, Rabbits and Rodents Clinical Medicine and Surgery, 2nd Edition. Elsevier: St. Louis.

Theodora.com. Biomethodology for Laboratory Mice: Blood Collection. http://www.theodora.com/rodent_laboratory/blood_collection.html

Section 5.2.8f. Appendix I. Supply and Equipment List

Note: Supply details, availability, and vendor sources may vary.

PPE

☐ Designated clothing (e.g. overalls or other clothes which can be put on before sampling and removed following sampling)
☐ Flexible face shield or other eye protection
☐ N95 or P100 respirator
☐ Nitrile examination gloves
☐ Washable shoes

First Aid

☐ Betadine or (or benzalkonium chloride)
☐ First aid kit

Data Collection

☐ Datasheets (or EIDITH tablet for direct data entry)
☐ Pencils
☐ GPS

Capture and Handling

☐ Sherman traps
☐ Flagging tape
☐ Leather gloves
☐ Holding bags
☐ Manual restrainer
☐ Spring/electronic balance
☐ Dial/digital caliper
☐ Nestlets or similar bedding material for traps
☐ Hand warmers or similar hypothermia treatment method
☐ Large/gallon-sized Ziploc-style (resealable) plastic bags
☐ Chemical restraint / anesthesia requirements
☐ Metal tea ball and/or nose cone for anesthesia maintenance, and/or isoflurane vaporizer
☐ 20ml plastic tubes
☐ Cotton balls
☐ Camera
☐ Identification guides
☐ Apples or other fruit (food with high water content) to help prevent animal dehydration

Sampling

☐ Clippers or scissors for hair / fur
☐ Glycerin or petroleum jelly
☐ Cotton-tipped swabs (for "combing" or clearing fur only, **NOT** for sampling)
☐ Processing trays

- ☐ Permanent lab markers for tube numbering
- ☐ Cryotubes
- ☐ Lancets
- ☐ Needles: 23G, 25G, 27G (Note: 20-22G needles may be used for facial bleeding after the technique has been mastered)
- ☐ Needles and syringes for blood draws
- ☐ Insulin syringe (27G-29G, x ½ inch, 1.0 ml) for fluid replacement
- ☐ Lactated Ringer's or 0.9% NaCl solution for fluid replacement
- ☐ Sterile swabs (dacron/polyester-tipped), appropriate sizes for sampling needs
- ☐ Sterile saline
- ☐ 2inch x 2inch gauze pads
- ☐ Povidone-iodine, 70% Isopropyl Alcohol, or Benzalkonium Chloride prep pads
- ☐ Silver nitrate styptic pencil
- ☐ Cryo resistant tube labels
- ☐ Cryovial rack
- ☐ Cryoboxes and dividers
- ☐ Plastic hematorit tubes
- ☐ Plastic vacutainers (EDTA and dry)
- ☐ Small vials or jars for tissue samples (necropsy sampling only)
- ☐ Pipetters and disposable tips
- ☐ Portable centrifuge for vacutainers
- ☐ Portable centrifuge for hematocrit tubes
- ☐ Cryo Gloves
- ☐ Fine Point Forceps
- ☐ Scissors
- ☐ Dissection kit
- ☐ 70% EtOH for sterilizing necropsy instruments
- ☐ Alcohol burner for sterilizing necropsy instruments and/or sterilizing scissors to cut swabs
- ☐ Trizol Reagent
- ☐ Viral Transport Medium (VTM)
- ☐ RNA Later
- ☐ Buffered formalin
- ☐ 95% ethanol
- ☐ Lighter
- ☐ Liquid nitrogen shipper/liquid nitrogen

<u>Waste Disposal and Decontamination</u>

- ☐ Paper towels
- ☐ Sharps containers
- ☐ Bleach
- ☐ 95% ethanol
- ☐ Biohazard bags
- ☐ Sprayers

Supply References

Goldenrod lancets: http://www.medipoint.com/html/for_use_on_mice.html

Microcapillary tubes with caps:
https://www.fishersci.com/us/en/catalog/search/products?storeId=10652&nav=122540&sortBy=default&keyword=Microvette+Capillary&searchType=PROD&SWKeyList=%5B%5D&typeAheadCat=

Microtainers with microgard closures: https://www.fishersci.com/shop/products/bd-microtainer-capillary-blood-collector-bd-microgard-closure-7/p-178764#tab2

Capillary blood collection tubes: https://www.fishersci.com/shop/products/terumo-capiject-capillary-blood-collection-tubes-8/p-178577

Nestlet bedding material: http://www.ancare.com/our-products/nestlets-ancare

Section 5.2.8g. Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines

a) To minimize contamination, choose the smallest fluid vial volume (i.e. 10mL) of Lactated Ringer's (preferred) or 0.9% NaCl solution. Warm the solution up to ~37^{o}C/100^{o}F.
b) Check the expiration date of fluid vial/bag. Visually inspect the vial integrity and fluid aspect (discoloration, haziness, crystallization, or particulate matters). Discard any contaminated, punctured or cracked vials/bags.
c) Select an injection site in the loose skin over the animal's neck or flank that has no evident skin nor tissue damage.
d) Wipe the chosen area with a 70% Isopropyl Alcohol or Benzalkonium Chloride prep pad.
e) Using – for small rodents – an insulin syringe (27G-29G, x ½ inch, 1.0 ml), insert the needle 5-10 mm through the skin before making the injection. Lack of resistance to the injection is indicative that you are in the right location.
f) Depending on individual size, inject subcutaneously 0.5-1ml (respectively for a juvenile and adult mouse) sterile fluid (see Figure below). Small rodents (~25gr) can safely receive up to 2-3ml subcutaneously and larger rodents (~200gr) up to 3-5ml.
g) Check for leaking from the injection site, especially if a larger volume is injected.
h) Discard syringe in sharps container.
i) You can draw multiple fluid doses from the same vial/bag used during the same sampling day. <u>Discard any opened fluid vial at the end of the sampling day</u>, <u>even if it is not empty</u>.

Replace fluids after collecting blood from rodents. Image © Leticia Gutiérrez Jiménez

Section 5.2.7. Bat Sampling Methods

Prepared by
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Matthew LeBreton, Metabiota
Melinda K Rostal, EcoHealth Alliance
and the PREDICT One Health Consortium

Objective: To safely collect biological samples from bats.

This document was made possible by the generous support of the American people through the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT program. It was drafted to support activities conducted under PREDICT and is intended for an audience of qualified professionals trained in standard, associated best practices. This guide is not intended for use by untrained individuals.

The authors assert that animal capture and sampling should always occur in compliance with all applicable laws and regulations and should only be undertaken after securing all necessary permits and approvals, including ethical approvals.

For more information about the contents of this guide, please contact predict@ucdavis.edu.

Suggested Citation Form: PREDICT One Health Consortium 2016. PREDICT Operating Procedures: Bat Sampling Methods.

Table of Contents: Bat Sampling

Section 5.2.7a. Confirmation of Knowledge

When you are familiar with the information in this Guide, take the PREDICT quiz ***Section 8.4.6. Bat Sampling***.

Section 5.2.7b. Brief Overview of PPE

All staff handling bats or their blood products should be vaccinated for rabies. If possible, ensure that they have a protective antibody titer, and be prepared to institute appropriate post exposure prophylaxis measures in the event that a bat bite or scratch occurs. It is recommended that personnel with frequent bat contact follow CDC and WHO guidelines of checking titers every two years (**www.who.int/rabies/WHO_Guide_Rabies_Pre_Post_Exposure_Prophylaxis_Humans_2013.pdf?ua=1**).

Minimum PPE required for handling live bats

The minimum PPE for handling bats during capture and sampling includes:

1. Eye protection
2. N95 or P100 respirator
3. Designated long-sleeved clothing
4. Nitrile gloves[1]
5. Washable shoes

(See the ***Biosafety and PPE Guide (Section 4.)*** for detailed instructions regarding PPE Use)

First aid protocol for a bite, scratch, or needlestick

1. The injured person must notify other research staff and work must stop.
2. The bite, scratch, or needlestick site should be washed well with water and betadine (povidone-iodine) or benzalkonium chloride (this is known to kill rabies virus) for a full 15 minutes. It is recommended that benzalkonium chloride be kept readily available in a first aid kit for such purposes.
3. If the injury (bite or scratch) is from a bat, the post-exposure rabies vaccination should be obtained as soon as possible. It is recommended that the field team develop a post-exposure vaccination plan with their physician prior to fieldwork if working in a remote location so that a booster dose can be administered soon after exposure. Otherwise, exposed personnel should immediately report to a medical clinic for administration of the booster doses. See WHO guidance for post-exposure prophylaxis at:
   http://www.who.int/rabies/human/postexp/en/
   http://www.who.int/rabies/human/prevvaccperson/en/

[1] Nitrile gloves are recommended for handling bats, in the absence of nitrile gloves and allergies to latex double latex gloves could be considered.

Section 5.2.7c. Data Collection

Please refer to the **required data collection templates** for data to collect. These include:

1. P2 Animal Data Collection Form
2. P2 Site and Event Characterization Data Collection Form
3. P2 Specimen Data Collection Form

For more information on downloading templates from EIDITH see ***Section 5.2.3. General Data Collection Templates and Applications***.

Section 5.2.7d. Bat Capture, Handling, and Sampling

Capture techniques will vary based on the species being targeted and the location where the samples are being collected and details of the main techniques including mist nets, harp traps, and hand capture are available in other documents such as the FAO guide *Investigating The Role Of Bats In Emerging Zoonoses* (**www.fao.org/docrep/014/i2407e/i2407e00.pdf**). Note that not all the sample collection techniques in that guide are recommended for PREDICT2 and field staff should use the PREDICT2 guide for specimen collection guidance.

Note*: The PPE requirements for handling animals during capture or during processing are the same. All animal capture, handling and sampling should be done in accordance with current IACUC protocols.*

Handling Procedures

1. Each bat should be placed into a porous cotton bag (with draw-string mouth), hung from a sturdy line over a polyethylene sheet (to catch urine), and kept in a cool dry place until sampling time.
2. Bats should be weighed (in grams) in bags using a Pesola hanging scale or a tabletop scale with or without a container (such as a cup). The container should be tared and both bat and bag should be weighed together. Once the bat is removed from the bag for sampling, the bag should be re-weighed and subtracted from previous total.
3. The bat should be removed from the bag and the samples below collected. The order of sampling may vary. For example, urine may be expelled on initial handling and urine would then be the first sample collected.
   Note: check bag for fresh feces before continuing. If fresh feces are available, these may be used as a sample and then a rectal swab is not necessary. The sampler must be certain that the feces belong to the bat being sampled. Bags should be either discarded after first use or washed/disinfected between uses.
4. Bats will not be held longer than 6 hours. Frugivorous and nectivorous bats will be give 100% fruit juice or sugar water prior to release.

Sampling Procedures

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two oral swabs** - one in 500 µL VTM and one in 500 µL Trizol
2. **Two fecal samples** - one with max of 500 µL/0.5cc feces in 500 µL VTM and one with max of 500 µL/0.5cc feces in 1 mL Trizol

   Or

   Two rectal swabs - one in 500 µL VTM and one in 500 µL Trizol
3. **Two blood samples** - 2 x 500 µL aliquots, one in 500 µL VTM and one in 500 µL Trizol
4. **Two serum samples** - 2 x 500 µL aliquots (only if more than 2ml of blood available), frozen without media. A minimum of 100 µL serum (single aliquot) should be collected to be useful for PREDICT diagnostic testing

***Note**: If animals are too small to collect two blood tubes (for whole blood and serum), collect serum and save remaining clot in VTM after serum separation*

5. **Two urogenital swabs/urine samples** - one with max of 500 µL of urine in 500 µL VTM and one with max of 500 µL of urine in 500 µL Trizol

Freeze all samples in liquid nitrogen immediately in the field and transfer to -80°C lab freezer.

If there is no **short-term** access (i.e., within 24 hours) to cold chain, such as in an emergency situation, then samples can be collected in 200 µL of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

- 1 day at 37 °C (i.e. ambient temp)
- 1 week in the refrigerator
- Within one week freeze at -80 °C for storage until analysis

Collection of samples from bats:

a. **Two oral swabs: 1 in Trizol, 1 in VTM**: Using sterile, polyester-tipped swabs with either an aluminum or plastic shaft, rub the swab tip gently but thoroughly against the back of the animal's throat, saturating the swab with saliva (recommend Puritan® Small Tapered Polyester-Tipped Swab from VWR, Catalog No.: 89133-756).
   i) Place 1 swab in a 1mL screw-top cryovial filled with 500 µL VTM and use alcohol-wiped (or ethanol-wiped), flame-sterilized scissors to cut the shaft of the swab about 1cm above the tip. Swabs should be cut on the shaft as close as possible to the end-swab without touching it. Scissors should be wiped with ethanol or isopropyl alcohol and flame sterilized after cutting each swab.
   ii) Place the other swab into 500 µL Trizol in a cryovial and cut the shaft as above.
   iii) Store in a liquid nitrogen dry shipper or dewar and transfer to -80˚C freezer later.

b. **Two rectal swabs: 1 swab in VTM, 1 swab in a tube with Trizol.** Rectal swabs can be moistened with sterile saline prior to animal sampling.

DO NOT USE VTM TO MOISTEN SWABS BEFORE SAMPLING
DO NOT USE TRIZOL AS A LUBRICANT *– IT IS HIGHLY IRRITATING TO TISSUE.*
DO NOT FORCE TIP OF SWAB INTO RECTUM*, IF IT WON'T ENTER EASILY, DO NOT COLLECT THIS SAMPLE.*

Gently insert the sterile swab tips, one at a time, into the animal's rectum. Place 1 swab in a cryovial filled with 500 µL of VTM and using isopropyl alcohol-wiped (or ethanol-wiped), flame-sterilized scissors cut the shaft of the swab above the tip (or snap as mentioned above). Place the other swab into a cryovial with 500 µL of Trizol. Store in a dewar or dry shipper with liquid nitrogen and transfer to -80˚C freezer when possible.

c. **Alternatively, collect fresh feces:** Add 500 µL or pea-sized pieces of feces directly into two vials, one containing 500 µL VTM (= maximum final ratio of 1:1) and one containing 1 mL Trizol (= maximum final ratio of 1:2) and mix each tube well. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80˚C freezer when possible.

d. **Whole blood in VTM and Trizol, and serum divided into two aliquots;**
   i) Manually restrain bats during blood collection. For larger bats, two or preferably three people are required for these manipulations: one person to safely restrain the bat, one to take samples, and a third to manage the tubes (i.e. unscrewing the lids, holding them up to the sample taker, making sure the lids are replaced tightly and kept in order) and record samples. Smaller insectivorous bats may be restrained and sampled by a single person. Anyone sampling bats should have had previous training in bat venipuncture to avoid injury to the animal. In addition:
      - It is recommended that large fruit bats (*Pteropus, Aceradon,* and other large species) be anesthetized using either injectable medetomidine (50 µg/kg) + ketamine (5 mg/kg) or gas anesthesia (isoflurane 4-5% induction, 2% maintenance).
      - The person restraining the bat is responsible for monitoring respiration and communicating respiratory status appropriately.
   ii) Bats must be bled with caution to maintain a ratio no greater than 10 µL of collected blood to 1 g of bat body weight (equivalent to 1% bodyweight).
   NOTE: for bats <100g we use the maximum amount of 6 µL per gram of body weight.
   iii) **For bats > 100 g**: Use a non-heparinized syringe to collect blood (not to exceed 1% of the total body weight). Recommended venipuncture sites include the propetagial (cephalic) vein, the uropetagial (saphenous) vein, or the brachial vein (Figure 1.) If volume allows, place some blood in an EDTA (lavender top) tube and some in a serum vacutainer (red-top) tube containing serum-clotting factor. From the lavender tubes collect 500 µL of whole blood and place in 500 µL VTM, and 500 µL of whole blood in 500 µL Trizol. After allowing the blood to clot in the red top tubes, either

spin tube in a centrifuge or allow tube to stand vertically on ice overnight. Use a sterile pipette tip and pipette gun to draw off serum and place even aliquots into 2 cryovials (minimum 60 µL).

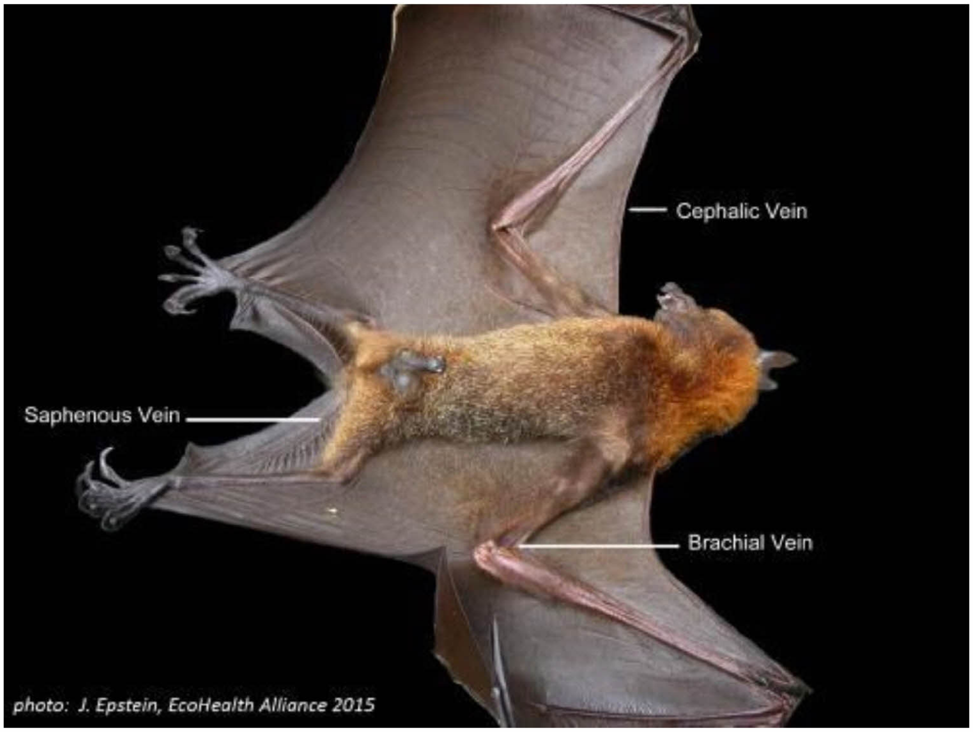

Figure 1. Preferred venipuncture sites for large (>100g) bats. Note: Apply pressure with a cotton ball to ensure that hemostasis is achieved after blood draw, especially with the brachial vein or artery, which are closely associated and higher pressure vessels compared to the cephalic or saphenous vein. (From Newman, Field, de Jong and Epstein, FAO 2011)

v) **For bats <100 g**: Use a 75 µL heparinized glass hematocrit tube to collect blood. Bat is restrained in one hand and the wing is gently extended by the wrist. The radial artery or vein is punctured using the tip of a sterile 25 G (gauge) needle and a droplet of blood is allowed to form. Collect up to 0.6% body mass of blood (e.g., 6µL per gram) using hematocrit tubes. Use a bulb to expel the whole blood in a cryotube with 500 µL of VTM. **Apply pressure to site of bleeding using a cotton ball until bleeding ceases (approximately 1 minute).** Hematocrit tubes can be centrifuged using a portable hematocrit centrifuge to separate serum. Score glass tube (using a razor blade or X-acto knife) where the serum meets the red cell fraction and carefully snap the tube. Use a bulb to expel serum into a micro-cryovial and freeze. If two or more capillary tubes are filled, collect two aliquots of serum. Preserve the remaining red cell clots in a separate cryovial and freeze.

vi) **Do not recap needle**. Place needle in sharps container and syringe in biohazard bag. Deliver medical waste to an incinerator or other secure medical waste disposal where possible.

vii) Bats must be fully recovered from anesthesia before release to prevent injury.

e. **Urogenital swabs/urine** - When handling bats, collect two urogenital swabs and place one into 500 µL VTM, one into 500 µL Trizol. Swabs can be moistened with sterile saline prior to animal sampling. If the bat urinates, collect two 500 µL urine samples at an optimal ratio of 1 part urine: 1 part VTM; and 1 part urine: 1 part Trizol (e.g., ~500 µL bat urine in 500 µL Trizol). Store samples in dry shipper or dewar with liquid nitrogen and transfer to -80˚C freezer when possible.
Note*: Larger fruit bats tend to urinate as they are removed from the cotton bag. Urine may be collected at this point using a pipette or tube. Urine may also be collected using a pipette from a surface however contamination is more likely and this should be avoided.*

f. **Necropsy sampling** - In case of accidental death before or during animal sampling, or where dead animals are available for opportunistic sampling, **collect tissue samples** – three, adjacent, approximately 200mg (pea-sized) pieces of each tissue type: one frozen in 500 µL VTM at -80°C, one frozen in 1 mL Trizol at -80°C, and one stored at room temperature, in a small vial or jar, in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment). In these cases also collect as much blood as possible. Cardiac puncture is recommended.

Collect approximately 200 mg (pea-sized) samples of the following tissues:

- Adrenal
- Colon
- Heart
- Liver
- Lymph node
- Ovary
- Testes
- Cecum
- Duodenum
- Kidney
- Lung
- Spleen
- Pancreas
- Other, if required

If euthanasia is required see the AAZV and ***AVMA guidelines (Section 8.5.2)***.

Additional Data To Collect:

Additional identification and biometric measurements may be collected at the discretion of the sampling party, although they are not mandatory (unless they are needed for species identification).

- Whole body photograph
- Identifying characteristic photographs
- Age class*
- Sex
- Body weight

- Body condition **
- Biometric measurements (see Biometrics section below for details)
- Additional morphometric measurements
- Reproductive status

*__Age classes__ - For some bat species it will be possible to classify bats into one of three age classes:

- **Pre-weaned juvenile** -- pup is still clinging to dam and suckling.
- **Juvenile** -- pup is independent from dam, may be adult sized, but sexually immature. Absence of secondary sexual characteristics such as elongated teats, not gravid at the time of capture, and for male Pteropodid bats, tiny barbules present on the glans penis. Incomplete fusion of phalangeal symphysis (head of phalanx not yet fused with shaft of phalanx as viewed when wing is backlit – this is more apparent with larger bats).
- **Adult** -- secondary sexual characteristics present, pregnant or lactating, adult size. Complete fusion of phalangeal symphysis.

**** Body Condition:** For larger fruit bats, it is also useful to evaluate body condition based on pectoral muscle mass--a quick and subjective measurement of nutritional status and robustness, which is a useful when assessing health in the context of infection. Record pectoral muscle mass as one of three categories: “Poor” (emaciated, prominent sternum), “Fair” (flat across pectoral muscles and sternum), “Good” (pectoral muscles are rounded and extend/bulge past the sternum).

Species identification:
1. The following digital photographs* should be taken of each bat where there is uncertainty about species identification:

a. Full body in anterior-posterior presentation and wings extended with identification card displaying unique identifying number
b. Full anterior facial (macro setting)
c. Full lateral facial/head (macro setting)
d. View of parted pelage on ventrum and dorsum (macro setting)

**Proper PPE should be worn at all times while holding animals, including while holding animals for photos or measurements.*

2. The biometric measurements (in millimeters) listed below should be taken. However, collecting these measurements adds time to the sampling effort. For micro-bats these are common measurements and they are valuable for identification; nevertheless the specific needs vary by species and by region. If you are in doubt of an identification look at reference texts for the genus or family and try to determine which the characteristics that

are relevant for that group. Someone with experience in identifying Microchiroptera in the area is usually required for this.

Microchiroptera biometric measurements **(as per Menzel et al., 2002)**

a. Forearm/radius length ('elbow to wrist')
b. Ear length (most distal tip of ear to middle of the base)
c. Tragus length (top of tragus to base of ear)
d. Body length (measured with the bat in ventral recumbancy from the tip of nose to the base of tail).
e. Hind foot length ('ankle to toe')
f. Tail length (from base to tip)
g. Tibia length ('knee to ankle')

Megachiroptera biometric measurements **(as per Menzel et al., 2002)**

a. Forearm/radius length ('elbow to wrist')
b. Head length
c. Body length

For larger fruit bats, it is also useful to evaluate body condition based on pectoral muscle mass--a quick and subjective measurement of nutritional status and robustness, which is useful when assessing health in the context of infection. Record pectoral muscle mass as one of three categories: "Poor" (emaciated, prominent sternum), "Fair" (flat across pectoral muscles and sternum), "Good" (pectoral muscles are rounded and extend/bulge past the sternum).

3. Based on these morphometrics and other appropriate unique characteristics, identify bats to genus, species (where possible), age class, and sex. For female bats, determine pregnancy status by gently palpating the abdomen and lactation status by gently attempting to express milk from the teats.

4. Release bats as close to their site of capture as possible.

5. If a sonic recording device is available, for Microchiroptera record the bat's calls upon release. These recordings can assist with identification of the specimens and with compiling resources for identifying bats in the area.

Section 5.2.7e. References

Food and Agriculture Organization of the United Nations. 2011. Investigating the role of bats in emerging zoonoses: Balancing ecology, conservation and public health interests. Edited by S.H. Newman, H.E. Field, C.E. de Jong and J.H. Epstein. FAO Animal Production and Health Manual No. 12. Rome. http://www.fao.org/docrep/014/i2407e/i2407e00.pdf

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Jonsson N. N., S.D. Johnston, H. Field, C. De Jong, and C. Smith (2004) Field anaesthesia of three Australian species of flying fox. *The Veterinary Record* 154:664.

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Menzel M.A., J. M. Menzel, S. B. Castleberry, J. Ozier, W.M. Ford and J.W. Edwards (2002) Illustrated Key to Skins and Skulls of Bats in the Southeastern and Mid-Atlantic States. USDA Forest Service, PA. 10p (Accessed 25.04.09 : http://www.fs.fed.us/ne/newtown_square/publications/research_notes/pdfs/2002/rnne376.pdf)

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Section 5.2.7f. Appendix I. Supply and Equipment List

Note: Supply details, availability, and vendor sources may vary.

PPE

- ☐ Designated clothing (e.g. overalls or other clothes which can be put on before sampling and removed following sampling)
- ☐ Flexible face shield or other eye protection
- ☐ N95 or P100 respirator
- ☐ Nitrile examination gloves
- ☐ Washable shoes

First Aid

- ☐ Betadine or (or benzalkonium chloride)
- ☐ First aid kit (with post-exposure prophylactic vaccine if working in remote areas where vaccine is not rapidly accessible)

Data Collection

- ☐ Datasheets (or EIDITH tablet for direct data entry)
- ☐ Pencils
- ☐ GPS

Capture and Handling

- ☐ Mist nets, poles and ropes
- ☐ Flagging tape
- ☐ Leather gloves
- ☐ Holding bags
- ☐ Spring/electronic balance
- ☐ Dial/digital caliper
- ☐ Stainless steel wing rulers
- ☐ Large ziplock bag
- ☐ Chemical restraint requirements
- ☐ Camera
- ☐ Identification guides

Sampling

- ☐ Processing trays
- ☐ Permanent lab markers for tube numbering
- ☐ Cryotubes

☐ Needles 25G, 27G
☐ Needles and syringes for blood draws
☐ Sterile swabs (dacron/polyester)
☐ Sterile saline
☐ Cryo resistant tube labels
☐ Cryovial rack
☐ Cryoboxes and dividers
☐ 75 µL glass hematocrit tubes (heparinized)
☐ Plastic vacutainers (EDTA and dry)
☐ Pipetters and disposable tips
☐ Portable centrifuge for vacutainers
☐ Portable centrifuge for hematocrit tubes
☐ Cryo gloves
☐ Fine point forceps
☐ Scissors
☐ Dissection kit
☐ Trizol reagent
☐ Viral Transport Medium (VTM)
☐ RNAlater reagent
☐ Buffered formalin
☐ 95% ethanol
☐ Lighter
☐ Liquid nitrogen shipper/liquid nitrogen

<u>Waste Disposal and Decontamination</u>

☐ Paper towel
☐ Sharps containers
☐ Bleach
☐ 95% ethanol
☐ Biohazard bags
☐ Sprayers

Section 5.2.11 Livestock Sampling Methods: Cattle, Sheep, Goats, Camels, and Swine

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Objectives: To safely collect biological samples from livestock.

This document was made possible by the generous support of the American people through the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT program. It was drafted to support activities conducted under PREDICT and is intended for an audience of qualified professionals trained in standard, associated best practices. This guide is not intended for use by untrained individuals.

The authors assert that animal capture and sampling should always occur in compliance with all applicable laws and regulations and should only be undertaken after securing all necessary permits and approvals, including ethical approvals.

For more information about the contents of this guide, please contact predict@ucdavis.edu.

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Table of Contents: Livestock Sampling Methods: Cattle, Sheep, Goats, Camels, and Swine

Section 5.2.11.a. Brief Overview of PPE

Minimum PPE Required for Livestock Sampling
The minimum PPE for livestock (including camels, cattle, sheep, goats, and swine) sampling includes:

1. Dedicated clothing
2. Nitrile (recommended) exam gloves
3. Safety glasses or other eye protection

(See the PREDICT ***Biosafety and PPE Guide (Section 4.1)*** for detailed instructions regarding PPE Use)

Standard disinfection procedures for equipment and clothing should be followed when moving between animal enclosures or properties.

Section 5.2.11.b. Livestock Handling and Welfare

Performance standards during handling include careful, considerate, respectful, calm, human interactions with animals in as positive a manner as is possible. Animals handled in a respectful manner will be calmer and easier to handle than animals handled in a rough or disrespectful manner. PREDICT field staff should be familiar with the correct techniques and the anatomy of each livestock species before attempting sampling procedures. At all times, observe animals for signs of excessive distress. If animals are unwell, stop all procedures, provide adequate support care, and release upon recovery.

While most veterinarians are familiar with handling livestock, we recommend that PREDICT staff visit the following guidelines as a refresher.
http://www.dardni.gov.uk/safe_cattle_handling_guidance.pdf

For more information on Animal Handling and Transport, see:
http://www.fass.org/docs/agguide3rd/Chapter05.pdf

For more information on welfare considerations for cattle handling, see:
http://www.animalwelfarestandards.net.au/files/2011/02/Cattle-Standards-and-Guidelines-for-Endorsement-May-0807141.pdf (Section 5, pages 13-16) and
Beef cows: **http://www.fass.org/docs/agguide3rd/Chapter06.pdf**
Dairy cows: **http://www.fass.org/docs/agguide3rd/Chapter07.pdf**

For more information on welfare considerations for sheep handling, see:
http://www.animalwelfarestandards.net.au/files/2011/02/Sheep-Standards-and-Guidelines-for-Endorsement-May-2014-080714.pdf (Section 5, pages 14 and 15) and
http://www.fass.org/docs/agguide3rd/Chapter10.pdf

For more information on welfare considerations for blood collection from cattle, see:
http://www.dpi.nsw.gov.au/agriculture/livestock/animal-welfare/general/livestock/sop/cattle/blood-collection

For more information on welfare considerations for swine handling, see:
http://www.fass.org/docs/agguide3rd/Chapter11.pdf

For more information on welfare considerations for camels handling, see:
http://www.publish.csiro.au/Books/download.cfm?ID=5204

Section 5.2.11.c. Sample Data Collection

Introductions and informed consent
Upon arriving to a household or farm, introduce yourselves (team members, purpose of the visit) to the acting head of household responsible for the livestock. Explain the purpose of the study, allow time for questions, and clarify any issues that may arise. If local regulations require it, obtain informed consent per project guidelines and protocols.

Animal Handling and Sampling Procedures
Note: For all food animals, manual restraint will be used. If drugs are used for sedation in a food animal, that animal will not be allowed to return the human food chain unless it is specifically labeled for use in that species and withdrawal periods are observed

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two nasal swabs** - one in 500 µL VTM and one in 500 µL Trizol
2. **Two fecal samples** - one with max of 500 µL/0.5cc feces in 500 µL VTM and one with max of 500 µL/0.5cc feces in 1 mL Trizol
   Or
   Two rectal swabs - one in 500 µL VTM and one in 500 µL Trizol
3. **Two whole blood samples** - one with max of 500 µL of whole blood in 500 µL VTM and one with max of 500 µL of whole blood in 500 µL Trizol
4. **Two serum samples** - 2 x 1.0 ml aliquots frozen without media
5. **Two urogenital swabs or urine samples** - one with max of 500 µL of urine in 500 µL VTM and one with max of 500 µL of urine in 500 µL Trizol

Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

If there is no **short-term** access (i.e. within 24 hours) to cold chain such as in an emergency situation, then samples can be collected in 500 µL of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

- 1 day at 37 °C (i.e. ambient temp)
- 1 week in the refrigerator
- Within one week freeze at -80 °C for storage until analysis

Collecting Nasal Swabs

Using sterile, polyester-tipped swabs with a plastic shaft, rub the swab tip gently but thoroughly against the walls of the animal's nares, about 1-2" from the opening, saturating the swab with mucus. **Place 1 swab in a cryovial filled with 500 µl of VTM and the other swab into 500 µL of Trizol in another cryovial**. Mix each tube well. Store both cryovials in a liquid nitrogen dry shipper or dewar & transfer to -80˚C freezer when possible.

Bleeding Collection Techniques

1. Cattle

Blood can be collected from the jugular vein in cattle of all ages or from the tail (coccygeal) vein of older cattle.

A variety of collection devices may be used - vacutainers, bleeding tubes, syringe and needle. Restraint should ensure quick, easy and safe collection of the sample causing minimal distress. This may involve use of a bail, race, or crush for tail bleeding. For jugular bleeding the animal may require minimal restraint (e.g. halter) or may need to be restrained in a crush with head bail and the employment of a halter or nose grips. Use of nose grips should be avoided wherever possible.

Operators should use gloves and disinfect or replace them between animals to prevent the transmission of blood-borne diseases. Equipment such as vacutainer holders should also be cleaned between animals. An antiseptic must be applied to clean skin surface prior to venipuncture.

For a visual guide see the following online tutorials:

Cattle
https://www.youtube.com/watch?v=IuNbsTMrIuI (tail and jugular)
https://www.youtube.com/watch?v=ZEsHMwKFbKg (tail)
https://www.youtube.com/watch?v=812CskWCqGQ (jugular)

Procedure for Jugular Venipuncture Using Vacutainer Needle and Tubes:

1. Identify and georeference the study site and document the signalment of the animal on the data collection sheet.
2. Before sample collection, ensure that the animal is effectively and humanely restrained to avoid injury to the animal and/or study personnel.

3. Using the halter, position the animal's head so that it is slightly elevated and drawn to the side opposite the jugular vein to be sampled.
4. Disinfect venipuncture area with alcohol
5. Occlude the vein by applying digital pressure in the jugular groove located in the lower neck.
6. Place a vacutainer needle attached to a vacutainer holder into the distended jugular vein at a 45° angle cranial to the jugular groove.
7. Once needle is positioned in the vein, insert a vacutainer into the needle to collect the blood.
8. When the desired volume has been collected (5 ml minimum suggested) remove the occluding pressure from the vein.
9. Detach the tube from the needle and withdraw the needle from the jugular vein.
10. You can collect more than 1 tube by repeating steps 7 and 8.
11. Label the vacutainer tubes with the sample ID.

Procedure for Jugular and Coccygeal Venipuncture Using Syringe and Needle

Jugular bleeding

1. Restrain cow with the head elevated and the jugular groove exposed.
2. Raise the jugular vein by placing pressure at the base of the jugular groove.
3. Pass the needle through the skin and into the vein by a firm thrust directed at an angle of 20^{o} to the skin surface.
4. Withdraw the blood sample.

Tail Bleeding

1. Restraint should prevent the cow from moving away during the procedure.
2. Raise the tail vertically with one hand until it is horizontal with the ground.
3. Approximately 150 mm from the base of the tail, locate the groove lying in the ventral midline of the tail.
4. Midway along the body of a coccygeal vertebra, insert the needle perpendicularly to the surface of the skin to a depth of a few millimeters.
5. Withdraw blood sample.
6. Apply pressure to the venipuncture site after withdrawal of the needle until the bleeding stops.

Once blood is collected, place the needle into a sharps container. Open red-top and purple top vacutainer tubes. Place approximately 2.5cc in each tube, then discard the syringe into a biohazard container. Invert each tube several times to mix.

2. Sheep/Goats

Blood should be collected from the jugular vein. The procedures for blood collection are identical to those described for cattle, with the exception of the amount of restraint needed and the possibility of shearing the bleeding area on the neck for easier viewing of the vein and minimizing the chance of introducing dirt or bacteria into the vein with the needle.

In sheep and goats, blood sampling can be done with assistance or alone. If you are not proficient at drawing blood alone, work with an assistant. The assistant should restrain the sheep/goat's body and turn the head to the side, at a 30-degree angle, by holding the animal under its jaw to allow for easy access to the jugular vein.

Restraining a sheep or goat without assistance is better for those who have become proficient at drawing blood. The handler should straddle the sheep/goat, place his or her knees behind the animal's shoulders, and back the sheep/goat into a corner or against a wall to help control their hindquarters. The sheep/goat's head should be turned opposite to the side of collection, once again at a 30-degree angle. Restraint of the head is accomplished by using the elbow and the upper arm to keep it held off to the side. This leaves both hands available for the blood collection.

The easiest way to locate the vein is to draw an imaginary line from the middle of the sheep/goat's eye down the side of the neck. The vein can be located by applying pressure with the thumb or fingers in the groove on either side of the trachea. The pressure will cause the vein to pop up and be easy to feel or see if the area has been shaved. Proceed as with cattle, using a vacutainer collection system or syringe and needle.

For a visual guide see the following online tutorials:

Sheep/goats (small ruminants) **https://www.youtube.com/watch?v=47tlmqXX3eE**

Blood sample processing and storage:

Whole Blood

- Collect whole blood into 1 lavender top tube containing EDTA, and allow another tube to clot for collection of serum.
- Add up to 500 μL of whole blood (from EDTA tube) directly into 2 vials, one containing 500 μL Trizol and one containing 500 μL VTM (= maximum final ratio of 1:1) and mix each vial well.

Serum

- After clotting is complete, use a plastic pipette to take 1 ml of serum and transfer into 2 cryovial tubes, 0.5 ml each.
- If a centrifuge is available, centrifuge samples for 15 minutes and then collect 1 ml serum and transfer into 2 cryovial tubes, 0.5 ml each.
- Label the cryovial tubes with the same label information used on vacutainer tube.
- You can harvest additional serum for serum bank as appropriate.
- Freeze all samples in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

3. Camels

Because of the risk of MERS CoV exposure, sample collectors should wear gloves, a respirator, and eye protection when handling camels.

Blood can be collected from the jugular vein in camels of all ages, though it is recommended that this be undertaken on animals while they are in sternal recumbency (kush position), well-restrained, or sedated. The lateral thoracic vein or caudal epigastric ("milk") vein may be used but should only be targeted in animals where physical or chemical restraint prevents kicking.

A vacutainer needle (18G or 19G) with purple top (EDTA) tubes and red-top (with serum clot activator) tubes may be used, or a 5cc syringe and 18G or 19G needle. Restraint should ensure quick, easy and safe collection of the sample causing minimal distress.

Equipment such as vacutainer holders should be cleaned between animals.

<u>Procedure for Jugular Venipuncture Using Vacutainer Needle and Tubes</u>

1. Identify and georeference the study site and document the signalment of the animal on the data collection sheet.
2. Before sample collection, ensure that the animal is effectively and humanely restrained to avoid injury to the animal and/or study personnel.
3. Using the halter, elevate the animal's head and draw it to the side opposite the jugular vein to be sampled.
4. Disinfect venipuncture area with alcohol
5. Occlude the vein by applying digital pressure in the jugular groove located in the lower neck. Alternatively, a rolled towel affixed with a rope over the withers can be applied at the same level to act as a temporary incomplete tourniquet.
6. Place a vacutainer needle, attached to a vacutainer holder, into the distended jugular vein at a 45° angle cranial to the jugular groove.
7. Once the needle is positioned in the vein, insert a vacutainer into the needle and collect the blood.
8. When the desired volume has been collected (5 ml minimum suggested), remove the occluding pressure.
9. Detach the tube from the needle.
10. Detach the needle from the jugular vein and apply pressure to the venipuncture site after withdrawal of the needle until the bleeding stops.
11. If more than one tube of blood is required, repeat steps 7 through 9 with occluding pressure.
12. Label the vacutainer tubes with sample ID.

Note: If vacutainer needles are unavailable, a 5cc syringe and 18G or 19G needle can be used. Once blood is collected, place the needle into a sharps container. Open red-top and purple top vacutainer tubes. Place approximately 2.5cc in each tube, then discard the syringe into a biohazard container. Invert each tube several times to mix.

Whole blood can be aliquoted into cryotubes with VTM and Trizol using a pipette gun. Serum tubes can either be centrifuged (if available) or placed vertically in a cooler with ice bricks and allowed to stand undisturbed overnight (~12 hours) for clean serum separation. Serum can then be aliquoted into cryotubes.

Procedure for Jugular Venipuncture Using Syringe and Needle

Jugular bleeding

1. Restrain camel with the head elevated and the jugular groove exposed.
2. Disinfect venipuncture area with alcohol
3. Raise the jugular vein by pressure at the base of the jugular groove.
4. Pass the needle through the skin and into the vein by a firm thrust directed an angle of 20° to the skin surface.
5. Withdraw blood sample.
6. Apply pressure to the venipuncture site after withdrawal of the needle until the bleeding stops.

Lateral Thoracic/Caudal Epigastric Vein Bleeding

1. Restraint should prevent the camel from moving away or kicking during the procedure.
2. Identify the lateral thoracic vein, caudal to the point of the elbow's olecranon process.
3. Pass the needle through the skin and into the vein by a firm thrust directed an angle of 20° to the skin surface.
4. Withdraw blood sample.
5. Apply pressure to the venipuncture site after withdrawal of the needle until the bleeding stops.

4. Swine

All personnel handling or sampling pigs should wear appropriate PPE and practice appropriate biosafety practices to avoid spreading infection from one animal to another and from one herd, farm or property to another. This includes wearing dedicated clothing (e.g. coveralls and rubber boots) that can be removed and disinfected once work at a site has been completed. Recommended PPE includes nitrile gloves, a respirator and safety glasses.

Restraint: Manual restraint is recommended, without the use of anesthesia. Pigs to be sampled should be constrained to a separate pen, if possible. The use of a snout snare (see appendix) by the animal restrainer is recommended for pigs over 20 kg, but should only be used by experienced personnel and for short term restraint to avoid injury to the pig's snout. Pigs will be restrained for a maximum of three minutes and then released. If blood collection is unsuccessful, then the pig will be allowed to calm down for five minutes before a second attempt is made.

Blood can be collected from the external jugular vein, or the cranial vena cava, using a 1", 20G needle and a 5cc syringe. This technique requires the head to be restrained and elevated parallel to the ground, typically using a snout snare. In pigs weighing less than ~50 kg, blood can be collected further caudally (and more medially) in the jugular groove, nearer the manubrium from anastomose of internal and external jugular vein. For pigs weighing less than ~20 kg, a technician will manually restrain the pig on his lap, holding the forelegs in one hand, and the animal's head in the other. Then a max of 5.0 to 10 ml may be collected from the jugular vein. Venipuncture should only be performed by experienced personnel.

The marginal ear veins are the only veins that are easily visible on pigs of any size. Usually there are three prominent veins. The lateral or central vein is usually the largest of these. These veins may also be punctured for blood collection. An alternative venipuncture site is the caudal auricular ("marginal ear") vein, though this typically yields low (<1 mL) blood volumes. A smaller, 22G or 23G needle should be used for this vein.

See also http://oslovet.norecopa.no/teaching/pig/pigbleed/ for more details on blood collection from pigs.

Collecting Fecal Samples

Ensure the animal is properly restrained prior to sampling. Fresh fecal samples should be collected, preferably from the rectum. If freshly passed, feces can be collected off the ground. Only the top part of a freshly passed fecal pat should be collected using a disposable spoon or scooped up in a gloved hand, plastic bag or plastic vial.

For Collection from the Rectum in Cattle and Camels

- The operator places an obstetrical sleeve on one arm
- The arm is formed into a cone and the animal's tail held to one side with the opposite gloved hand.
- Gentle pressure is applied to the anal sphincter until penetration into the rectum is obtained.
- A fecal aliquot of sufficient size for the intended laboratory procedure is scooped with the sleeved hand and removed from the animal.
- The fecal sample is placed in a separate container or the obstetrical sleeve is inverted off the arm such that the fecal sample is trapped inside.

Small calves, sheep, goats, and swine: restrain manually. Gently pass a gloved, lubricated finger through the anus and massage the rectal wall to stimulate rectal evacuation. If feces are not produced, collect feces with finger.

Place two ~200 mg (pea size) samples of fresh feces into 2 vials, one containing 500 µL VTM (= maximum final ratio of 1:1) and one containing 1 mL Trizol (= maximum final ratio of 1:2). Homogenize by shaking. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80˚C freezer when possible.

If feces are not available, collect 2 rectal swabs- 1 in VTM and 1 in Trizol: Gently insert one sterile swab tip at a time into the animal's rectum. [Note: DO NOT USE TRIZOL AS A LUBRICANT – IT IS HIGHLY IRRITATING TO TISSUE.] Place 1 swab in a cryovial filled with 500 µL of VTM. Place the other swab into a tube with 500 µL of Trizol. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80˚C freezer when possible.

Collecting Urine/Urogenital Swabs

Many animals will urinate as a fear reaction while they are handled. Urine can be collected free catch in plastic vials. Add up to 500 µL of urine directly into 2 vials, one containing 500 µL VTM and one containing 500 µL Trizol (= maximum final ratio of 1:1) and mix each tube well. Store in dry shipper or dewar with liquid nitrogen and transfer to -80˚C freezer when possible.

If urine is not available, collect 2 urogenital swabs: 1 in VTM and 1 in Trizol. Place 1 swab in a cryovial filled with 500 µL of VTM. Place the other swab into a tube with 500 µL of Trizol. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80˚C freezer when possible.

Section 5.2.11.d. Sample Collection from Dead or Euthanized Livestock

PREDICT's primary approach to sample collection in livestock is to collect specimens from living animals. In the event that an animal has died of natural causes or been euthanized due to humane or veterinary care reasons, the guidelines below for necropsy sampling may be followed. If bodies are relatively whole and fairly fresh, then sample as described above. The *American Veterinary Medical Association guidelines (Section 8.5.2.)* in the PREDICT Operating Procedures ebook provides information on animal euthanasia that may be useful to PREDICT veterinarians called upon to euthanize an animal.

As discussed throughout this protocol, all animals should be considered potentially infectious for a wide variety of dangerous pathogens, and dead animals in particular should be sampled only following all safety measures, including proper PPE use, proper work station decontamination, and proper carcass disposal, as outlined here and in other PREDICT documents.

Though not required for PREDICT sampling, thorough necropsy procedures can be very beneficial and relevant for some animals (e.g., suspicious deaths). Time and skill permitting, when full necropsies are performed, following any Association of Zoos and Aquariums/AZA (or similar) necropsy protocol is recommended and most can be adjusted for application to livestock species. Necropsy protocols are also addressed in the Non-Human Primate Sampling protocol, Appendix V.: AAZV's Occupational Primate Disease Safety Guidelines for Zoological Institutions: Standardized Necropsy Report for Non-Human Primates Work Sheet (ebook Section 5.2.6j.); most of the information and worksheets in this document can be utilized for sampling of livestock. (*Note that properly following extensive necropsy procedures and collecting and measuring all samples can require 4-6 hours for a single animal.*)

Duplicate blood samples are to be collected from each animal; one sample must be collected into Trizol and one into viral transport media (VTM). If only one sample can be collected, then place the sample into VTM.

Tissue specimens should be collected in triplicate. One specimen should be frozen in 500 μL VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).

Post-Mortem Blood Collection

From recently dead animals, it may be possible to collect whole blood (often clotted) from the right side of the heart where the largest volume of blood is available. Collect all available blood into an appropriate size container (typically one or more blood tubes). Allow the tubes to sit undisturbed for at least 30 minutes, and then centrifuge at high speed (2000 x G for 20 minutes). Transfer the serum (clear, yellow or red-tinged fluid at the top), preferably via pipetting, to appropriately labeled cryovials. Transfer the remaining blood clots to separate cryovials. Refrigerate or freeze both the serum and blood clots.

If a centrifuge is not available, allow the clots and cells to settle as much as possible, and then collect the serum and clots as described above. If the animal's death is recent enough that the blood has not yet clotted and a centrifuge is not available, invert the blood tubes after the blood has been collected to allow the clot to form on the rubber stopper. After the blood has clotted, turn the tube right side up and carefully remove the stopper with the adhered clot, thereby leaving a clean serum sample in the tube.

At a minimum, as many of the following blood samples as possible should be collected:

- 2 samples of 500 μL (**whole blood**) placed in 2 vials, one containing 500 μL **Trizol** and one containing 500 μL **VTM** (= maximum final ratio of 1:1). Mix each vial well.
- 2 or more aliquots (0.5 ml) of **separated serum**, frozen

Tissue Collection

Collect three, adjacent, approximately 200mg (pea-sized) samples of the following tissues:

- Adrenal
- Colon
- Heart
- Liver
- Lymph node
- Ovary
- Testes
- Cecum
- Duodenum
- Kidney
- Lung
- Spleen
- Pancreas
- Other, if required*

*It will usually require experience to identify abnormal tissues, but potentially recognizable gross lesions include masses, discolored areas, ulcerations, etc. Samples for histopathology (i.e., in formalin) should be collected at the abnormal margins to include both normal and abnormal sections in the same piece of tissue. Collection of any obvious internal parasites in ethanol is also recommended.

Section 5.2.11.e. References

Higgins, A. J., & Kock, R. A. (1984). A guide to the clinical examination, chemical restraint and medication of the camel. *The British Veterinary Journal*, *140*(5), 485–504.

Fowler, M. E. (2010). Chapter 4 Clinical Diagnosis: Examination and Procedures in *Medicine and Surgery of Camelids*. (3 edition). Ames, Iowa: Wiley-Blackwell.

http://www.dardni.gov.uk/safe_cattle_handling_guidance.pdf

http://www.fass.org/docs/agguide3rd/chapter05.pdf =

http://www.dpi.nsw.gov.au/agriculture/livestock/animal-welfare/general/livestock/sop/cattle/blood-collection

http://www.biotracking.com/goats/biopryn/use

Section 5.2.11.f. Appendix I. Dentition Age Determination for Cattle, Sheep, and Goats

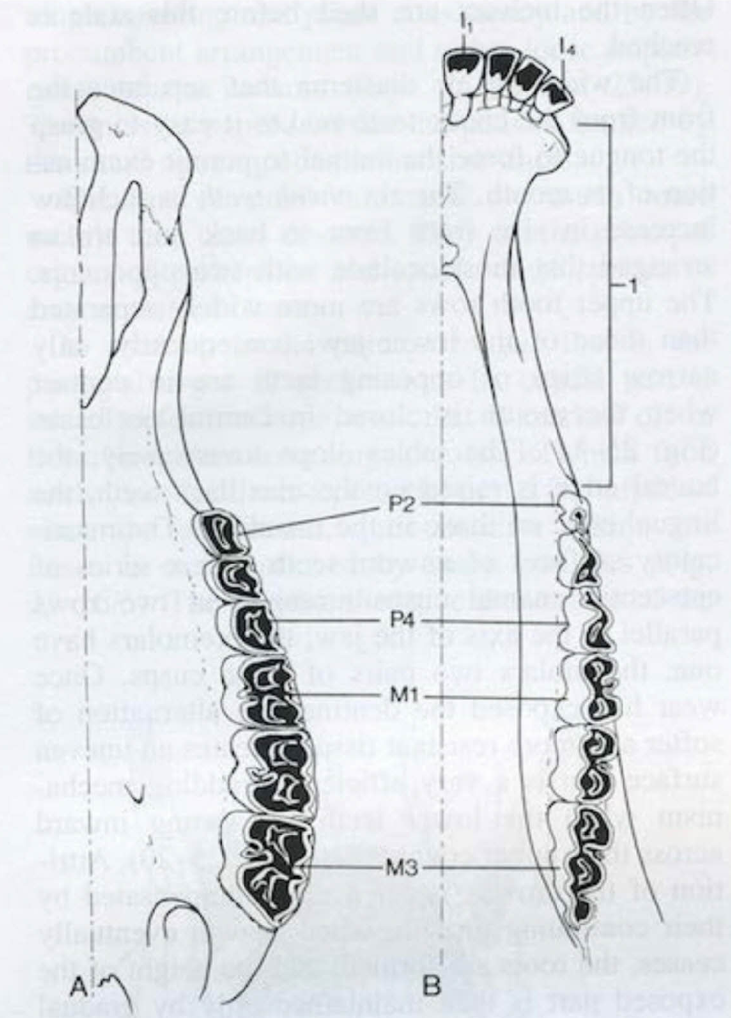

FIGURE 25–20. Left half of upper and right half of lower jaw of cattle. Notice the different shapes of the upper and lower cheek teeth and the large diastema (1).

Figure 1: From Dyce, Keith M., Wolfgang O. Sack, and Cornelis Johannes Gerardus Wensing. Textbook of Veterinary Anatomy. Elsevier Health Sciences, 2009.

Table 1: Eruption dates of the teeth of cattle

Teeth	Deciduous Teeth	Permanent Teeth
Incisor 1	Birth to 2 weeks of age	18 – 24 months
Incisor 2	Birth to 2 weeks of age	24 – 30 months
Incisor 3	Birth to 2 weeks of age	36 months
Incisor 4	Birth to 2 weeks of age	42 – 48 months
Premolar 2	Birth to 1 week	24 – 30 months
Premolar 3	Birth to 1 week	18 – 30 months
Premolar 4	Birth to 1 week	30 – 36 months
Molar 1		12 – 18 months
Molar 2		24 – 30 months
Molar 3		18 – 24 months

Table 2: Eruption dates of the teeth of sheep and goats.

Teeth	Deciduous Teeth	Permanent Teeth
Incisor 1	Birth to 1 weeks of age (at birth)	12 – 18 months
Incisor 2	Birth to 1 weeks of age (at birth)	18 – 24 months
Incisor 3	Birth to 1 weeks of age (at birth)	30 – 36 months
Incisor 4	1 to 3 weeks	36 – 48 months
Premolar 2	3 weeks	18 – 24 months
Premolar 3	3 weeks	18 – 24 months
Premolar 4	3 weeks	18 – 24 months
Molar 1	3 – 4 months	
Molar 2	8 – 10 months	
Molar 3	18 – 24 months	

Section 5.2.11.g. Appendix II. Snares


Figure 1: A commercial snout snare (left) and use of a modified snout snare, made from local materials, to restrain a pig during sampling in Bangladesh (right).

Section 5.2.11.h. Appendix III. Checklist for Supplies

General equipment and supplies

- ☐ Animal handling equipment – Halters and animal restraining ropes
- ☐ Data Collection forms
- ☐ Rubber stamp ink and pad
- ☐ GPS
- ☐ Camera
- ☐ Field Notebook
- ☐ Pen/Pencil
- ☐ Permanent markers
- ☐ Cryomarkers
- ☐ Protective clothing – Waterproof rubber boots, overalls, facemask, and nitrile gloves
- ☐ First aid kit
- ☐ Ice box containing ice packs (for short term storage and transport)
- ☐ Sharps bin
- ☐ Sturdy garbage bags
- ☐ Field centrifuge (portable 12vt)
- ☐ Liquid nitrogen dewar

Blood sample collection equipment and supplies

- ☐ EDTA vacutainer tubes – 9ml (lavender top)
- ☐ Serum separator vacutainer tubes – 9ml (red/gray top)
- ☐ Vacutainer needle holders
- ☐ Vacutainer needle: Cattle and Camels, 1½" 18 or 19 gauge; Sheep, Goats, and Swine, 1" 20G
- ☐ Syringes: 20, 10 and 5 ml
- ☐ Needles: Cattle and Camels, 1½" 18 or 19 gauge; Sheep, Goats, and Swine, 1" 20 guage for jugular or 22 or 23 gauge for auricular vein
- ☐ Alcohol (squirt bottle or vaporizer)
- ☐ Gauze
- ☐ Vacutainer tube rack
- ☐ Cryovial tubes
- ☐ Cryovial rack
- ☐ Centrifuge
- ☐ VTM
- ☐ Trizol

Fecal Sample Collection Equipment and Supplies

- ☐ Obstetrical Sleeve
- ☐ Disposable Spoons
- ☐ Plastic bags or vials
- ☐ Cryovial Rack
- ☐ Cryovials with VTM and Trizol

Urine Sample Collection Equipment and Supplies

☐Plastic vials
☐Plastic pipettes
☐Cryovial Rack
☐Cryovials with VTM and Trizol

Swab Collection Equipment and Supplies

☐Plastic handle, polyester tip swabs
☐Cryovial Rack
☐Cryovials with VTM and Trizol

Tissue Collection Equipment and Supplies (in case of animal necropsy)

☐ 21 Gauge needles for cardiocentesis
☐ 1 mL Syringe for cardiocentesis
☐ Scalpel and surgical blades
☐ Forceps
☐ Sharp and blunt tip scissors
☐ Cryovial Rack
☐ Cryovials with VTM and Trizol
☐ Small Vials or Jars
☐ 10% Buffered Formalin

Section 5.2.11.i. Appendix IV. Additional Permit Requirements for Livestock Samples Imported into the United States

In addition to all other permits, livestock samples require special import permits from the USDA.

http://www.aphis.usda.gov/publications/plant_health/2012/fs_imp_food_ppq.pdf

http://www.aphis.usda.gov/wps/portal/aphis/resources/permits/!ut/p/a1/jZDLDoIwFES_hi0dKmJ1VyVCfUVjjNi
NQYOVBKgpKL8vGjfG5-
zuzTnJZIgkEZFFfElVXKW6iLPbLb2tKxilLVDBlgsPYjiftQeUOgi8Btg0wCDgoduZAHAZhfD7od_pTgHh_efjQzh--
Wsin5HA4X7jLSezRTgExs4D-FbxDnzpMCJSZXp332PDi12LKSJNckhMYuyzad7HqjqVPQsW6rq2ldYqS-
y9zu3YWHhnHXVZkegFJqd8FSGd52tW8ivrt9DV/?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa
_our_focus%2Fsa_animal_health%2Fsa_import_into_us%2Fct_animal_imports_home

From:	Andrew Clements <aclements@usaid.gov>
To:	Jonna Mazet <jkmazet@ucdavis.edu>;Murray, Suzan <MurrayS@si.edu>;Christine Kreuder Johnson <ckjohnson@ucdavis.edu>;Tracey Goldstein <tgoldstein@ucdavis.edu>
CC:	Pereira, Alisa (GH/HIDN) <apereira@usaid.gov>;Cara Chrisman <cchrisman@usaid.gov>;Shana Gillette <sgillette@usaid.gov>;David J Wolking <djwolking@ucdavis.edu>
Sent:	8/1/2017 2:27:28 AM
Subject:	Request for information on lab testing results from Myanmar

Hi all,

In an e-mail today regarding the status off H1N1 infections in people and H5N1 in poultry in Myanmar, the Mission included the following sentence: "While pending formal announcement, PREDICT/Smithsonian representatives indicated that bat samples in the Yangon area exhibit H5N1 infection."

Since this would be a first (as far as I am aware), can you confirm that this is correct? If it is correct, can you provide additional information on when and where samples were collected? Has the government been officially notified and presented with the proper context and risk assessment?

If the information is not correct, we need to squash this rumor.

Thanks!

Andrew

--

Andrew Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
E-mail: aclements@usaid.gov

For more information on USAID's Emerging Pandemic Threats program, see: http://www.usaid.gov/ept2

From: [redacted] on behalf of "Jonna Mazet" <jkmazet@ucdavis.edu>
Sent: 08/09/2017 9:41:50 AM (-07:00)
To: "PREDICTMGT" <predictmgt@usaid.gov>
Subject: Fwd: [predict] H1N1 updates of Myanmar, July 25

FYI,
J

---------- Forwarded message ----------
From: **Ohnmar Aung** <[redacted]>
Date: Wed, Aug 9, 2017 at 7:59 AM
Subject: Re: [predict] H1N1 updates of Myanmar, July 25
To: Jonna Mazet <jkmazet@ucdavis.edu>
Cc: PREDICT-outbreak <Predict-outbreak@ucdavis.edu>, "predict@ucdavis.edu" <predict@ucdavis.edu>, "Murray, Suzan" <[redacted]>, "Zimmerman, Dawn" <[redacted]>, "Valitutto, Marc" <[redacted]>, kyaw tun <[redacted]>

Dear Dr. Mazet,

Thank you for your kind inquiry into the status of the recent H1N1 outbreak in Myanmar.

In brief, the Ministry of Health and Sports (MOHS), has declared the outbreak, deemed “a seasonal flu,” to be under control, following the guidance of the WHO and other partner health organizations in the country. There is no current need or active request for PREDICT assistance at this time.

The Myanmar CDC provided an update summary yesterday (8 Aug) stating as of now, 60 patients remain hospitalized, all of whom are stable and recovering. In total for the outbreak: 433 patients were hospitalized with severe respiratory infection, 166 were found to be H1N1 positive, and 17 patients expired due to severe pneumonia and secondary complications.

The Deputy Director General, Dr. Htay Htay Tin, of the National Health Laboratory (NHL) has confirmed that the current H1N1 outbreak is the same as the original strain which we have seen seasonally (wet season) since 2010.

Last Tuesday, 1 Aug, our PREDICT team was invited to partake in an update and discussion session regarding the status of H1N1 and H5N1 outbreaks. Led by WHO, other attendants included USAID Health Director, Global Fund, CDC, Japanese Embassy, JICA, UN agencies, and FAO. Understanding that the H1N1 outbreak exhibits a seasonal trend, the main objective of this meeting was to address public anxiety in addition to a request for PCR kits and laboratory reagents. Due to the panic, there has been a shortage of supplies at NHL for testing.

Regarding the H5N1 outbreak in chickens, the FAO has been working closely with the Livestock Breeding and Veterinary Department (LBVD). 3000-5000 chickens had died in the Tanintharyi region (southern Myanmar) and close to 2000 more birds were culled, effectively controlling the outbreak in the region. An additional isolated event was reported to the OIE from an event on 26 Jul in the Yangon region involving a small number of village owned chickens (22). These specimens were killed and disposed of. No further cases have been reported to our knowledge. No crossover to humans has been identified.

Thank you again for your continued attention to our PREDICT Myanmar Project.

Sincerely,

Ohnmar

Dr Ohnmar Aung

Project Coordinator | PREDICT | Myanmar | **Global Health Program**

REDACTED

On Wed, Aug 9, 2017 at 7:00 AM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:

Hi Ohnmar,

Thanks again for the info and expressing our willingness to support. Was there any follow-up? We will be discussing tomorrow on our Executive Board call, so if there is anything else to share, please advise,

Jonna

On Tue, Jul 25, 2017 at 9:56 AM, Ohnmar Aung <REDACTED> wrote:

Dear PREDICT Global,

We wanted to provide you with an update regarding the H1N1 concern in Myanmar.

Here is today's update of H1N1 in Yangon General Hospital from Myanmar CDC. (25 July 2017)

- Out of 12 suspected flu cases in YGH, three found to be Seasonal Influenza A H1N1 (pdm2009) positive through nasopharyngeal swab testing in National Health Laboratory.

- Out of these 12 suspected cases, 25 years old, male, admitted on 21 July with severe pneumonia and expired on 24 July.

- Another case of a 29 year old pregnant woman who was hospitalized in Central Women Hospital with severe pneumonia and transferred to YGH in the morning on 24 July and expired in the afternoon of same day.

With the reference of US CDC, morality rate is 0.02% and seasonal flu with complications is 4.55%, therefore, MOHS warned the community against panicking. MOHS will be providing all necessary information and educational facts to the community through different channels of media including social media.

I already informed PREDICT's interest to CEU's colleagues but no response yet.

Also, a news report surfaced yesterday re: H5N1 with subsequent culling of birds in the Tanintharyi region (far south Myanmar) on July 16th. This report follows the news of the H1N1 detection in the Yangon General Hospitals. While we work closely with the Ministry of Livestock, this report has yet to be shared with our PREDICT team, although we have inquiries to our ministry counterparts. We are also pending a response from our counterparts in FAO who works with the livestrock ministry for avian influenza testing.

http://www.reuters.com/article/us-health-birdflu-myanmar-idUSKBN1AA136

Thank you,

Ohnmar

Dr Ohnmar Aung

Project Coordinator | PREDICT | Myanmar | **Global Health Program**

REDACTED

From: "Ehab Abu-Basha" <abubasha@just.edu.jo>
Sent: 09/21/2017 4:19:34 AM (-07:00)
To: "William B. Karesh" <karesh@ecohealthalliance.org>; "Jon Epstein" <epstein@ecohealthalliance.org>
Cc: "Suzan Murray" <murrays@si.edu>; "David Wolking" <djwolking@ucdavis.edu>; "Zimmerman, Dawn" <Zimmermand@si.edu>; "Patrick Dawson" <dawson@ecohealthalliance.org>; "Peter Daszak" <daszak@ecohealthalliance.org>; "Simon Anthony" <anthony@ecohealthalliance.org>; "Tracey Goldstein" <tgoldstein@ucdavis.edu>; "Prof. Woutrina Smith" <wasmith@ucdavis.edu>; "mohamed ali" REDACTED "Amanda Andre" <amanda.andre@ecohealthalliance.org>; "Kirsten Gilardi" <kvgilardi@ucdavis.edu>; "Kevin Olival, PhD" <olival@ecohealthalliance.org>; "Aleksei Chmura" <chmura@ecohealthalliance.org>; "Ghazi Kayali" <ghazi@human-link.org>; "Jonna Mazet" <jkmazet@ucdavis.edu>; "Predict inbox" <predict@ucdavis.edu>
Subject: Re: High Priority - need MERS related info for WHO/FAO meeting
Attachments: Report for Bat samples.docx

Dear Billy,

I am sorry for the delay on response but I was out for a field trip.

- Total bat samples no to date: 560 BATS
HUMAN SAMPLES: 115

221 BATS WERE TESYED FOR 4 VIRAL FAMILIES. THE REST ARE IN PROCESS.
All positives were sent to Princess Hiya biotechnology center (PHBC) for sequencing. (result in the attached file)

- Laboratory Diagnostic were available for analyses performed on camel samples from Azraq, Ramtha, and Aqaba, which were collected during May 2016 sampling operation by the PREDICT Jordan team with FAO, Kansas State University, and the National Institute of Health (NIH)'s Rocky Mountain Laboratories. All tests were performed at the JUST Faculty of Veterinary Medicine Laboratory. Nasal swab samples were tested for MERS-CoV RNA using real-time polymerase chain reaction (PCR). Of the 120 samples tested, 36 were positive for MERS-CoV. Of the 36 positive samples, 22 (61%) were from Al-Ramtha in northern Jordan, and 14 (39%) were from Azraq near the Saudi Arabian border. The results were reported to the Ministry of Agriculture, which provided them to OIE for immediate notification. Another 24 samples were collected from another farm in Ramtha and were tested against MERS-CoV using RT-PCR and found negative. **The results were reported to MOA and they reported to OIE. A manuscript was submitted for publication and recently was accepted for publication in vector borne and zoonotic disease journal. This demonstrates a positive collaboration between several organizations and a successful story.**

Best regards,

Ehab

Ehab Abu-Basha, DVM, MSc., Ph.D
Professor of Pharmacology and Toxicology
Faculty of Veterinary Medicine
Jordan University of Science and Technology

REDACTED

From: William B. Karesh <karesh@ecohealthalliance.org>
Sent: Tuesday, September 19, 2017 4:04 PM
To: Jon Epstein
Cc: Ehab Abu-Basha; Suzan Murray; David Wolking; Zimmerman, Dawn; Patrick Dawson; Peter Daszak; Simon Anthony; Tracey Goldstein; Prof. Woutrina Smith; mohamed ali; Amanda Andre; Kirsten Gilardi; Kevin Olival, PhD; Aleksei Chmura; Ghazi Kayali; Jonna Mazet; Predict inbox
Subject: Re: High Priority - need MERS related info for WHO/FAO meeting

Dr. Ali has 15 minutes, so I would suggest:

1) Country
2) Taxon sample targets
3) numbers of individuals sampled
4) relevant results (I'm not sure what we say about "MERS-like CoV's, but please include if something interesting to share).

I know that for Jordan and Egypt we are doing integrated surv. around MERS with FAO, but I'm not sure how best to describe Ethiopia, Kenya, Uganda and Tanzania. Or the Asian countries.

Thanks everyone.

BK

William B. Karesh, D.V.M
Executive Vice President for Health and Policy

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President, OIE Working Group on Wildlife

Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

On Sep 19, 2017, at 8:50 AM, Jon Epstein <epstein@ecohealthalliance.org> wrote:

Billy,
How much detail do you want? Sample numbers, or more general info about where and what we're testing?

-Jon

Jonathan Epstein DVM, MPH, PhD

Vice President for Science and Outreach

EcoHealth Alliance
New York

(e) epstein@ecohealthalliance.org
(o) 212.380.4467
(m) REDACTED
@epsteinjon

On Sep 19, 2017 8:38 AM, "William B. Karesh" <karesh@ecohealthalliance.org> wrote:
Hi Everyone,

WHO asked Dr. Ali, our PREDICT Country Coordinator for Egypt, to give a presentation on PREDICT's MERS-CoV work in camels.   I let WHO know that we are looking at MERS along with MERS-like CoV's in humans, wildlife and camels.

This will be the only PREDICT presentation at the meeting.

In the next day or two, could you please share with Dr. Ali (and Patrick can help compile) what you doing or our findings with camels, humans, wildlife in:

Uganda
Tanzania
Kenya
Ethiopia
Egypt
Jordan
Bangladesh
Thailand

Is there anything to add from China??
Other countries??

Thanks,

Billy

William B. Karesh, D.V.M
Executive Vice President for Health and Policy

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New York, NY 10001 USA

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EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

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All positives were sent to Princess Hiya biotechnology center (PHBC) for sequencing.

Table 1.a: Summary of the RT-PCR results for the bat samples collected from Wadi-Alseer, Amman, Jordan (100 samples).

	Paramyxo		Influenza		Filo		Corona	
	Oral	Rectal	Oral	Rectal	Oral	Rectal	Oral	Rectal
Wadi Elseer Bat no. 1-100	0	0	4	3	0	0	7	35

Table 1.b: Summary of the RT-PCR results for the bat samples collected from Ajloun, Jordan (121 samples).

	Paramyxo		Influenza		Filo		Corona	
	Oral	Rectal	Oral	Rectal	Oral	Rectal	Oral	Rectal
Ajloun Bat no. 1-121	0	0	2	0	0	0	21	36

Sequences of coronaviruses were blasted to either:

BtRf-AlphaCoV/HuB2013 (GenBank: KJ473807)

Rousettus bat coronavirus/Kenya/KY06/2006 (GenBank: HQ728483)

From: "Ehab Abu-Basha" <abubasha@just.edu.jo>
Sent: 09/21/2017 4:34:00 AM (-07:00)
To: "William B. Karesh" <karesh@ecohealthalliance.org>
Cc: "Jon Epstein" <epstein@ecohealthalliance.org>; "Suzan Murray" <murrays@si.edu>; "David Wolking" <djwolking@ucdavis.edu>; "Zimmerman, Dawn" <Zimmermand@si.edu>; "Patrick Dawson" <dawson@ecohealthalliance.org>; "Peter Daszak" <daszak@ecohealthalliance.org>; "Simon Anthony" <anthony@ecohealthalliance.org>; "Tracey Goldstein" <tgoldstein@ucdavis.edu>; "Prof. Woutrina Smith" <wasmith@ucdavis.edu>; "mohamed ali" REDACTED "Amanda Andre" <amanda.andre@ecohealthalliance.org>; "Kirsten Gilardi" <kvgilardi@ucdavis.edu>; "Kevin Olival, PhD" <olival@ecohealthalliance.org>; "Aleksei Chmura" <chmura@ecohealthalliance.org>; "Ghazi Kayali" <ghazi@human-link.org>; "Jonna Mazet" <jkmazet@ucdavis.edu>; "Predict inbox" <predict@ucdavis.edu>
Subject: Re: High Priority - need MERS related info for WHO/FAO meeting
Attachments: vector born and zoonotic diseases paper.pdf

Thanks Billy. Please find enclosed the published paper.

See you soon.

Ehab

Ehab Abu-Basha, DVM, MSc., Ph.D
Professor of Pharmacology and Toxicology
Faculty of Veterinary Medicine
Jordan University of Science and Technology
REDACTED

From: William B. Karesh <karesh@ecohealthalliance.org>
Sent: Thursday, September 21, 2017 2:25 PM
To: Ehab Abu-Basha
Cc: Jon Epstein; Suzan Murray; David Wolking; Zimmerman, Dawn; Patrick Dawson; Peter Daszak; Simon Anthony; Tracey Goldstein; Prof. Woutrina Smith; mohamed ali; Amanda Andre; Kirsten Gilardi; Kevin Olival, PhD; Aleksei Chmura; Ghazi Kayali; Jonna Mazet; Predict inbox
Subject: Re: High Priority - need MERS related info for WHO/FAO meeting

Thanks Dr. Ehab!! Great work.

BK

Sent from my iPhone

William B. Karesh, D.V.M
Executive Vice President for Health and Policy

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New York, NY 10001 USA

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EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

On Sep 21, 2017, at 1:19 PM, Ehab Abu-Basha <abubasha@just.edu.jo> wrote:

Dear Billy,

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Ehab Abu-Basha, DVM, MSc., Ph.D
Professor of Pharmacology and Toxicology
Faculty of Veterinary Medicine
Jordan University of Science and Technology
[redacted]

From: William B. Karesh <karesh@ecohealthalliance.org>
Sent: Tuesday, September 19, 2017 4:04 PM
To: Jon Epstein
Cc: Ehab Abu-Basha; Suzan Murray; David Wolking; Zimmerman, Dawn; Patrick Dawson; Peter Daszak; Simon Anthony; Tracey Goldstein; Prof. Woutrina Smith; mohamed ali; Amanda Andre; Kirsten Gilardi; Kevin Olival, PhD; Aleksei Chmura; Ghazi Kayali; Jonna Mazet; Predict inbox
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3) numbers of individuals sampled
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-Jon

Jonathan Epstein DVM, MPH, PhD

Vice President for Science and Outreach

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New York

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On Sep 19, 2017 8:38 AM, "William B. Karesh" <karesh@ecohealthalliance.org> wrote:

Hi Everyone,

WHO asked Dr. Ali, our PREDICT Country Coordinator for Egypt, to give a presentation on PREDICT's MERS-CoV work in camels. I let WHO know that we are looking at MERS along with MERS-like CoV's in humans, wildlife and camels.

This will be the only PREDICT presentation at the meeting.

In the next day or two, could you please share with Dr. Ali (and Patrick can help compile) what you doing or our findings with camels, humans, wildlife in:

Uganda
Tanzania
Kenya

Ethiopia
Egypt
Jordan
Bangladesh
Thailand

Is there anything to add from China??
Other countries??

Thanks,

Billy

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<Report for Bat samples.docx>

VECTOR-BORNE AND ZOONOTIC DISEASES
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SHORT COMMUNICATION

High Prevalence of Middle East Respiratory Coronavirus in Young Dromedary Camels in Jordan

Neeltje van Doremalen,[1] Zaidoun S.K. Hijazeen,[2] Peter Holloway,[3] Bilal Al Omari,[4] Chester McDowell,[5] Danielle Adney,[6] Hani A. Talafha,[4] Javier Guitian,[3] John Steel,[7] Nadim Amarin,[8] Markos Tibbo,[9] Ehab Abu-Basha,[4] Ahmad M. Al-Majali,[4] Vincent J. Munster,[1,*] and Juergen A. Richt[5,*]

Abstract

Prevalence of Middle East respiratory syndrome coronavirus (MERS-CoV) was determined in 45 dromedary camels from two geographically separated herds in Jordan. Virus shedding was only detected in swabs obtained from the respiratory tract and primarily observed in camels younger than 3 years. MERS-CoV seroprevalence increased with age of camels. Bovine and sheep sera were seronegative. Phylogenetic analysis of partial S2 clustered the Jordanian MERS-CoV strains with contemporary MERS-CoV strains associated with nosocomial outbreaks.

Keywords: dromedary camel, Jordan, MERS-CoV, phylogeny, serology

Introduction

Since the identification of Middle East respiratory syndrome coronavirus (MERS-CoV) as the causative agent of a fatal case of respiratory tract disease in the Kingdom of Saudi Arabia (KSA) in 2012, the virus has caused >1700 laboratory-confirmed cases of disease, including >600 fatal cases (WHO 2016). The first known MERS-CoV outbreak in humans, diagnosed retrospectively, occurred in Jordan in 2012 (Hijawi et al. 2013). The dromedary camel has been identified as the primary reservoir of MERS-CoV, and direct evidence for zoonotic transmission from camels has been reported in KSA and Qatar (Haagmans et al. 2014, Memish et al. 2014). A limited number of seropositive camels in Jordan have been described previously (Reusken et al. 2013), but no MERS-CoV has been detected in camels in Jordan.

Materials and Methods

During May 2016, we collected swabs (from nasal, urogenital, and rectal areas) and blood samples from camels at two locations in Jordan. Two camel herds were identified to study (1) a traditional bedouin camel herd, in which camels are allowed to graze and browse freely (Azraq, Zarqa) and (2) a more conventional mixed farm setting, where camels were kept in pens on one farm (Ramtha) (Fig. 1A). In Ramtha, blood samples from adult sheep and cattle were additionally collected. Swab samples were collected in virus transport medium. RNA was extracted from samples using the QiaAmp Viral RNA kit (Qiagen). Five microliters of RNA was used in a one-step real-time RT-PCR UpE assay for MERS-CoV using the Rotor-Gene™ probe kit (Qiagen). Positive samples (cycle threshold [Ct] < 37) were tested using the ORF1A assay (Corman et al. 2012), and samples were excluded from further analysis when ORF1A testing was negative. cDNA was synthesized using random hexamer and used to PCR amplify the MERS-CoV spike S2 domain (nucleotides 23781–24395 of HCoV-EMC/2012) as described previously (Smits et al. 2015). Sequences were assembled on SeqMan Pro and analyzed on MegAlign (DNASTAR). Phylogenetic trees of the S2 domain were generated using Mega 6.0.6 with the maximum likelihood statistical method

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[7]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia.
[8]Boehringer Ingelheim, MENA, Dubai, United Arab Emirates.
[9]FAO Regional Office for the Near East and North Africa, Cairo, Egypt.
*These authors contributed equally to this work.

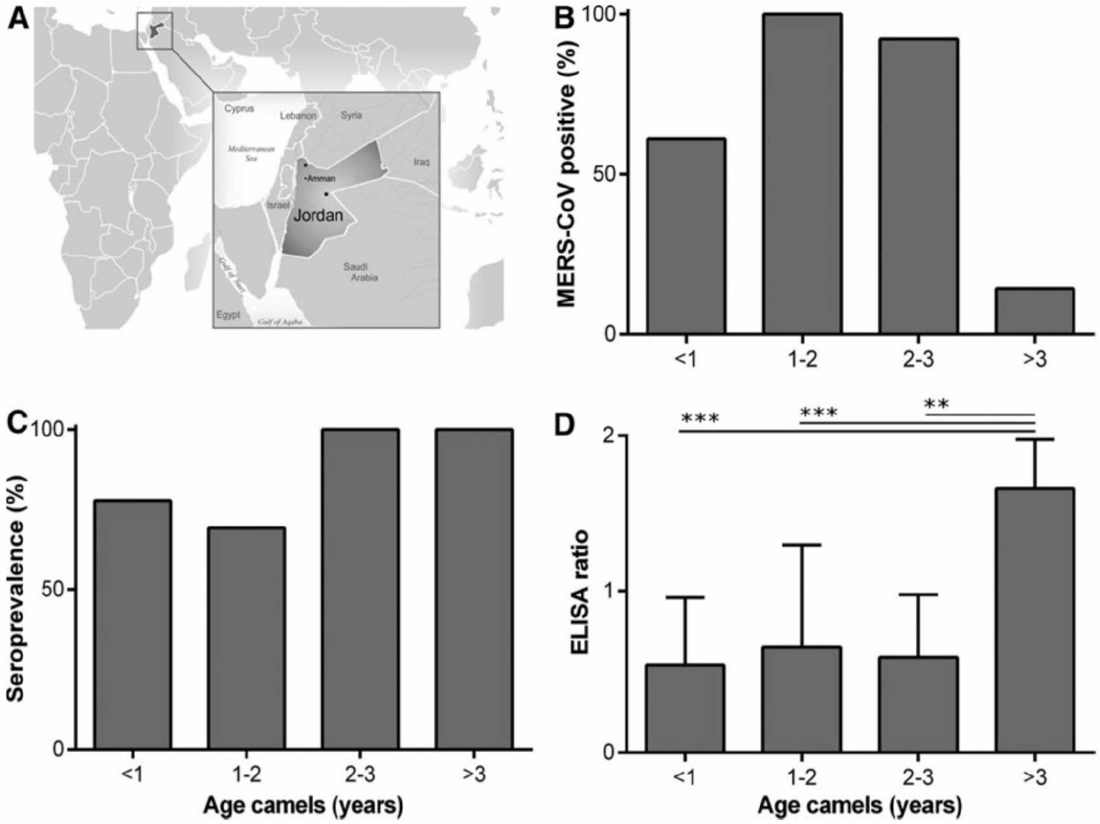

FIG. 1. MERS-CoV prevalence in dromedary camels in Jordan. **(A)** Locations of dromedary camels (north: Ramtha; east: Azraq). **(B)** Percentage of MERS-CoV RNA shedding dromedary camels as detected by UpE and ORF1A qRT-PCR assay in nasal swab, stratified by age. **(C)** Percentage of MERS-CoV S1-specific seropositive dromedary camels, stratified by age. **(D)** ELISA ratio of seropositivity of dromedary camels, stratified by age. The ELISA ratio was calculated by dividing the OD of each serum sample by a constant positive sample on the ELISA plate. ELISA, enzyme-linked immunosorbent assay; Middle East respiratory syndrome coronavirus (MERS-CoV), OD, optical density.

based on the GTR+G+I model with 1000 bootstraps replicates. Sera were analyzed by MERS-CoV spike protein (S) enzyme-linked immunosorbent assay (ELISA); Maxisorp (Nunc) plates were coated overnight with S1 protein (Sino Biological) and blocked with 1% milk. Sera (400× dilution) were added to the plate in duplicate. MERS-CoV S1-specific antibodies were detected using anti-llama (Agrisera), anti-bovine, or anti-sheep (Jackson) IgG (H&L) HRP-conjugated antibody on development with peroxidase-substrate reagent (KPL); optical density (OD) was measured at 405 nm. The threshold of positivity was mean OD + 10× standard deviation of negative sera obtained from camels raised in captivity in the United States.

Results

Twenty-three camels sampled from the Bedouin herd in Azraq ranged in age from 4 months to 8 years, whereas 22 camels sampled at the farm in Ramtha ranged in age from 4 months to 3 years (Table 1). Ten sheep and five cows were sampled. Nasal discharge was observed at the time of sampling in some of the MERS-CoV-positive camels <1 year old but not in older camels.

RNA from 42/45 camels was tested positive for the presence of MERS-CoV nucleic acid. MERS-CoV RNA was solely detected in nasal swabs. Importantly, urogenital and rectal swab samples were negative. One nasal swab originating from camel 40 was excluded from analysis; while the UpE assay resulted in a Ct value of <37, the ORF1A assay was negative. An unpaired two-tailed Student's *t*-test comparing the age of viral RNA-positive versus RNA-negative camels was significant ($p = 0.0311$). Only 1/7 camels older than 3 years was positive for viral RNA, whereas 11/18 camels <1 year old, 4/4 camels 1–2 years old, and 12/13 camels 2–3 years old were positive for viral RNA in nasal swabs (Fig. 1B). MERS-CoV-specific antibodies were observed in the majority of animals: 78% of camels <1 year old, 69% of camels 1–2 years old, and 100% of camels >2 years old were seropositive (Fig. 1C). No MERS-CoV S1-specific antibodies were found in sheep or bovine serum samples.

To better assess the potential significance of ELISA values as a correlate of MERS-CoV susceptibility, we calculated the ratio of the ELISA value of each sample to that obtained from an included camel reference serum on each plate in the assay. By this approach, we show that camels <3 years old exhibited an average ELISA ratio of 0.5–0.65, whereas older animals exhibited an average ELISA ratio of 1.63. This difference was significant as tested via a two-tailed Mann–Whitney test (Fig. 1D). Spike S2 partial domain sequences were obtained from 16/28 samples (accession

Table 1. Age, Location, and Assay Results Per Dromedary Camel

Camel	Age	Herd	Virus positive	TCID50eq/swab UpE	TCID50eq/swab ORF1A	Seropositive	ELISA ratio
40	2M	Ramtha	0			1	1.15
41	2M	Ramtha	0			1	0.50
42	2M	Ramtha	1	135	156	0	0.11
07	4M	Azraq	1	12	37	0	0.18
13	4M	Azraq	1	15	93	0	0.19
33	4M	Ramtha	1	252	196	1	0.17
36	4M	Ramtha	0			1	0.79
09	5M	Azraq	1	200	728	1	0.43
12	5M	Azraq	1	21	35	0	0.21
16	5M	Azraq	0			1	0.35
35	5M	Ramtha	1	92	109	1	0.29
06	6M	Azraq	1	81	264	1	0.30
17	6M	Azraq	1	188	174	1	1.27
24	6M	Ramtha	0			1	1.43
34	6M	Ramtha	0			1	0.43
37	6M	Ramtha	1	2729	1256	1	0.30
10	7M	Azraq	0			1	0.58
11	7M	Azraq	1	70	209	1	1.07
25	12M	Ramtha	1	9575	4420	0	0.13
26	13M	Ramtha	1	1569	2643	0	0.09
30	14M	Ramtha	1	5139	6054	0	0.13
32	14M	Ramtha	1	5907	2760	0	0.09
18	15M	Azraq	1	56	802	1	1.46
08	18M	Azraq	1	76	86	1	1.25
14	18M	Azraq	1	128	371	1	1.05
15	18M	Azraq	1	23	119	1	1.35
27	18M	Ramtha	1	1067	1074	1	0.16
28	18M	Ramtha	1	507	876	1	0.15
29	18M	Ramtha	1	168	368	1	0.63
31	18M	Ramtha	1	491	230	1	0.21
21	19M	Azraq	0			1	1.79
38	30M	Ramtha	1	280	452	1	1.10
39	30M	Ramtha	1	152	155	1	0.54
44	2Y	Ramtha	1	112	157	1	0.57
45	2Y	Ramtha	1	76	219	1	0.15
01	3Y	Azraq	0			1	1.76
20	3Y	Azraq	0			1	1.74
43	3Y	Ramtha	1	87	179	1	0.82
02	4Y	Azraq	0			1	1.83
04	5Y	Azraq	0			1	1.85
22	5Y	Azraq	0			1	1.89
03	6Y	Azraq	ND			1	1.76
19	6Y	Azraq	0			1	1.23
05	8Y	Azraq	ND			1	1.81
23	8Y	Azraq	ND			1	1.67

ELISA ratio was defined by dividing the OD of each serum sample by a constant positive sample on the ELISA plate. All viral RNA was detected in respiratory tract swab samples, none in urogenital or fecal swab samples. Positivity is marked as "1," negativity as "0."
ELISA, enzyme-linked immunosorbent assay; M, month; OD, optical density; Y, year.

numbers KX443663–KX443678). We were unable to obtain sequences of 12 positive samples with a lower viral load (Ct >33). We performed phylogenetic analysis with a selection of MERS-CoV S2 sequences using representatives of known clades, as performed previously (Smits et al. 2015). The phylogenetic analysis of the partial S2 sequences placed the novel Jordanian viruses within the B1 cluster representing contemporary camel and human MERS-CoVs. Three sequences were identical to spike S2 sequences of human isolates of MERS-CoV obtained in Jordan in 2015 (Lamers et al. 2016). Twelve samples differed by two synonymous mutations (C23837T, T24074G), whereas the remaining samples contained a mixture of 24074T and 24074G combined with 23837T. Importantly, all novel sequences were found in cluster B1, containing the most recent MERS-CoV sequences originating from camel and human viruses (Fig. 2). Thus, the circulating MERS-CoV strains in the Jordan dromedary camel population are closely related to virus strains known to be capable of zoonotic transmission and to cause disease in the human population.

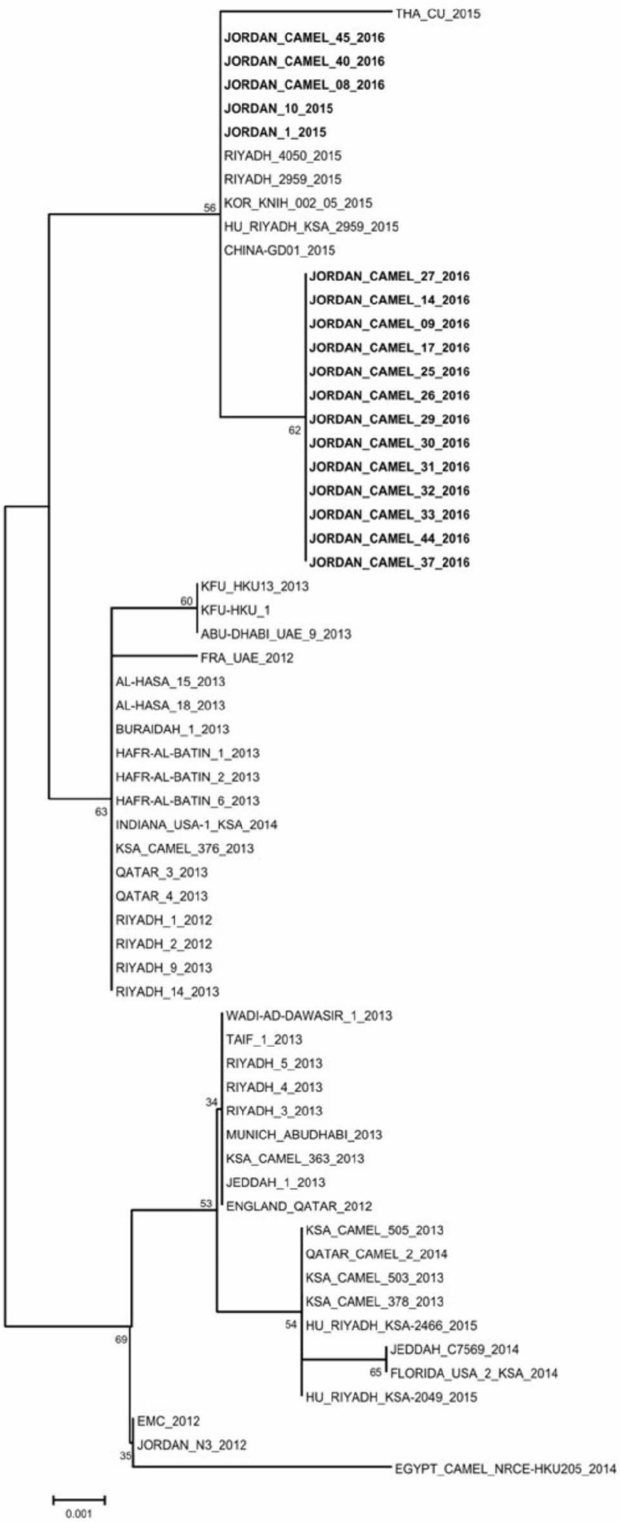

FIG. 2. Phylogenetic analysis of a partial spike S2 domain. A maximum likelihood tree based on the GTR+G+I model using 1000 bootstraps was generated from a spike S2 domain genome fragment corresponding to nucleotides 23781–24395 of HCoV-EMC/2012. The newly identified MERS-CoV sequences are depicted in *bold*, recent MERS-CoV sequences associated with an outbreak in Jordan in 2015 are depicted in *bold*. Bootstrap values of <50 were not shown.

Discussion

This study confirms the circulation of MERS-CoV within the dromedary camel population of Jordan in line with MERS-CoV detection in camels throughout the Middle East (Reusken et al. 2016). Importantly, MERS-CoV RNA could be detected in nasal swabs of seropositive dromedary camels. Antibodies against MERS-CoV were found at a young age in dromedary camels. The presence of maternal antibodies via the intake of colostrum during the first 24 h postparturition could play a role in the detection of MERS-CoV-specific antibodies in animals <6 months old and might not reflect actively acquired antibodies (Kamber et al. 2001, Meyer et al. 2016).

We observed shedding of MERS-CoV in the presence of antibodies, which suggests either reinfection of seropositive animals or shedding of virus/viral RNA during early stages of seroconversion. Previously, a lack of correlation was observed between virus/viral RNA shedding and the presence of neutralizing antibodies; these data highlight the potential for reinfection of seropositive animals (Farag et al. 2015). The ELISA ratio might be a better predictor of MERS-CoV susceptibility than seropositivity; 24/29 animals with an ELISA ratio of <1.1 were MERS-CoV positive, whereas only 4/16 animals with an ELISA ratio of ≥1.1 were positive for viral RNA in nasal swabs. In contrast, 20/34 seropositive dromedary camels were MERS-CoV viral RNA positive versus all (8/8) seronegative dromedary camels. This might indicate that sterile immunity is only reached at high levels of antibody titers, in line with a previous study conducted in the United Arab Emirates (Meyer et al. 2016).

The phylogenetic analysis of the partial S2 sequences placed the circulating viruses identified in the camel population within the B1 cluster representing contemporary dromedary and human MERS-CoVs. The clustering with human MERS-CoVs known to have caused nosocomial outbreaks in the KSA, South Korea, and Jordan underlines the zoonotic potential of these camel-derived MERS-CoVs.

Conclusions

While the most recent nosocomial outbreaks in Jordan were linked to travel-related cases from KSA as reported by the WHO, the detection of B1-cluster-like MERS-CoV in dromedary camels indicates that local introductions of MERS-CoV into the human population is a real possibility in Jordan, in addition to introductions via travel-associated cases.

Acknowledgments

This research was supported in part by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, the Jordan University of Science and Technology project 272/2015, internal support from Emory University, the Food and Agriculture Organization of the United Nations' component of the USAID-funded Emerging Pandemic Threats phase 2 (EPT-2) Program and the Kansas Bioscience Authority. We want to sincerely thank Boehringer Ingelheim for logistic assistance provided before and during research, and Austin Athman for assistance with figures.

Author Disclosure Statement

No competing financial interests exist.

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From: predict-request@ucdavis.edu on behalf of "Molly Turner" <turner@ecohealthalliance.org>
Sent: 11/29/2017 8:36:18 AM (-08:00)
To: "David J Wolking" <djwolking@ucdavis.edu>
Cc: "Evelyn Luciano" <luciano@ecohealthalliance.org>; "Ava Sullivan" <sullivan@ecohealthalliance.org>; "William Karesh" <karesh@ecohealthalliance.org>; "Peter Daszak" <daszak@ecohealthalliance.org>; "Alison Andre" <andre@ecohealthalliance.org>; "Amanda Andre" <amanda.andre@ecohealthalliance.org>; "Matt Blake" <mblake@ucdavis.edu>; "predict@ucdavis.edu" <predict@ucdavis.edu>
Subject: [predict] Re: Urgent: PREDICT ceiling increase questions from USAID
Attachments: Liberia field disposables_$52K final.xlsx, liberia supplies narrative_final.docx

Sounds good, we'll be ready.

Here's the narrative for the $52K.

Molly

On Wed, Nov 29, 2017 at 10:55 AM, David J Wolking <djwolking@ucdavis.edu> wrote:

> Thanks Molly, received. We'll be in touch with any questions today.
>
> D
>
> On Wed, Nov 29, 2017 at 6:06 AM, Molly Turner <turner@ecohealthalliance.org> wrote:
>
>> My apologies, attached is the correct Liberia field disposables sheet.
>>
>> On Wed, Nov 29, 2017 at 9:00 AM, Molly Turner <turner@ecohealthalliance.org> wrote:
>>
>>> Hi David,
>>>
>>> Please see below.
>>>
>>> Molly
>>>
>>> 8. No detail is provided for the large $52,473.00 lump sum cost for "field disposables" under the EHA subagreement "supplies" line item. Please provide a description of items to be purchased, number, and unit cost. In addition, there is an inconsistency between the figure stated in the budget ($52,473) and the figure in the budget narratives ($152,473). Please confirm the correct figure and adjust either the budget or narratives as appropriate.
>>>
>>> This is referring to "field disposables" for Liberia. We made an error the narrative where we state "...field disposables (for 2,500 animals/year) $152,473" so apparently an extra "1" was added to the $52,473 that is actually in the budget. If you can get us some breakdown of this $52,473 and how it relates to the 2,500 animals that would be super!
>>>
>>> As we discussed yesterday, we actually budgeted for 10,000 animals per year in the narrative and budget we submitted, for a total of $195,831 in Year 4. I checked again this morning and it says this on page 80 of the narrative that Liz returned to us as what was submitted to USAID (attached). I don't see $52,473 anywhere. Are you sure this is what we should provide?
>>>
>>> As we agreed I have justified $52,473 for 2,500 animals in the attached excel spreadsheet. I am working on the narrative, which I plan to model on the India portion of what Liz returned to us (page 53). Would be great if you could confirm that this is what you need as soon as you can.

9. EHA proposed a 35.4% indirect cost rate. Please provide a copy of the most current NICRA so that the rate and bases of application may be verified.

Can you just share your latest PDF of the NICRA? Liz says it was in the original packet from 7/24/17 but we should just attach it again (and I don't have it on hand) for quick reference.

Attached is the NICRA we provided.

10. Please provide a justification for NPHIL's 60% indirect cost rate. Please provide audited financials, a current NICRA or other appropriate justification.

This has to be an error (unless NPHIL has a NICRA or other documentation for that high rate). In our Y4 budget for Liberia (the recently approved one) it's listed as 10% (deminimus rate for a foreign sub without a NICRA per the uniform guidance), so we just need a confirmation here.

We agreed on yesterday's call that this 60% was probably drawn from CU's rate, for which they can provide you with documentation upon request. NPHIL is only budgeted for a 10% de minimus rate.

11. No fringe is proposed for NPHIL Liberia staff. Please confirm that this is in accordance with local law or adjust your budget accordingly.

Did you build the fringe or benefits rates into their salaries (Lab technicians 1-4 at $6,800, $7000, $7200, and $7,400)? Any insight here appreciated.

Liberian law only requires that we provide salary.

12. EHA proposed a total of $325,000.00 for Dr. Desmond, a consultant who will serve as Country Coordinator for their Liberia program (lump sum amounts: $125,000 Year 3, $125,000 Year 4, and $62,500 Year 5). Please provide Dr. Desmond's proposed daily rate, number of days EHA proposes him to work per program year, and information regarding whether he is an expatriate or local hire.

In the budget narrative we have Jim at a $125,000 rate for Y3 and 4 and then $62,500 in Y5. This breaks down to about $520/day at a 20 day per month rate (what UCD uses for consultants). That would be 1,120 days/year. He's a US citizen correct so expat? How do we explain the Y5 reduction in LOE and that rate? Any insight here appreciated as well in addition to corrections to my crude calculations above :-)

Jim's rate is actually hourly ($65/hour) and is competitive for a field veterinarian of his experience, especially since this rate needs to cover salary, benefits, and some supplies and local travel. $520/day would be correct, but I calculate that this comes to 240 (8-hour) days/year. He is an expatriate.

We did not actually decrease him in Year 5, but this could be reasonable since field work will be reduced or completed at that point, so less of his time will be required.

On Tue, Nov 28, 2017 at 4:32 PM, David J Wolking <djwolking@ucdavis.edu> wrote:

Thank you!!!

🙏

On Tue, Nov 28, 2017 at 1:27 PM, Molly Turner <turner@ecohealthalliance.org> wrote:
Hey David,

Received, we'll have for you by OOB tomorrow, and will give you a call if we have any questions.

Molly

On Tue, Nov 28, 2017 at 4:05 PM, David J Wolking <djwolking@ucdavis.edu> wrote:

Hey Molly, Evelyn, and team,

We finally received feedback from USAID's AO today about the ceiling increase. The bad news is they asked for a one day turnaround on their questions. Jonna, Matt, and I are pulling together all that we can to prepare for a call tomorrow afternoon.

There are a few questions related to EHA and your subs that we need feedback on ASAP, by OOB tomorrow (west coast) at the latest. Hopefully these are not that difficult and please just call me on my cell REDACTED if you have any questions.

Apologies for the tight turnaround but considering how long it has taken to get this far, we think it's in our best interest to work with them while they are focused on our award.

Thanks for understanding,

David

EHA-related feedback from the AO (David's comments in blue):

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--
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www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

--

Molly Turner
Federal Grants Coordinator

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

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EHA has budgeted for the purchase of field disposables associated with the capture of 2,500 bats as follows:

Bat capture disposables include cotton bat bags, bait mix and Nestlet (Ancare) cotton nesting material for thermoregulation.
Y4:
Bat bags (cotton), $1.00 per unit, 198 units ($198)
Bait mix (Peanut butter, nuts, rolled oats, seeds, apple slice) $5.00 per unit, 250 units ($1250)
Nestlet (Ancare) cotton nesting material for thermoregulation, $247.00/box of 3,600, one box ($247.00)

Blood collection disposables include hematocrit tubes (50mm and 75 mm), hematocrit tube sealant, bulbs to fit hematocrit tubes, cryovials, caps, cotton balls, alcohol swabs, needles, vacutainers, pipette tips, syringes, sharps containers, viral transport media, and swabs.
Y4:
Hematocrit tubes (microtubes), 50mm no heparin, $160.00/case of 1000, 4 cases ($640)
Hematocrit tubes (microtubes), 75mm no heparin, $134/case of 1000, 4 cases ($536)
tube sealant, $500/case, 1.25 cases ($625)
Bulb to fit hematorcrit tubes, $4.00/unit, 27 units ($108)
Cyrovials 0.5mL, $372.00/case of 500, 27 cases ($10,044)
Corning internally threaded cryovials, color caps 1.2 mL, $372.00/case of 500, 27 cases ($10,044)
Corning internally threaded cryovials, color caps 2.0 mL, $372.00/case of 500, 27 cases ($10,044)
Wheaton 0.5mL cryovial, $243.00/case of 500, 27 cases required, ($6,561)
Needles 27g ¾, $100.00/case of 1000, 2 packs ($200)
Needles 25g 3/4, $100.00/case of 1000, 2 packs ($200)
Needles 23g ¾, $100.00/case of 1000, 2 packs ($200)
Needles 18g ¾, $100.00/case of 1000, 2 packs ($200)
Red-top vaccutainer 3.0 mL, $182.00/case of 1000, 3 cases ($546)
Red-top vaccutainer 6.0 mL, $206.00/case of 1000, 1 case ($103)
Pipette tips 1000 µl, $120.00/case of 960, 2 cases ($240)
Sterile filtered pipette tips 100 µl, $100.00/box of 96 tips, 2 boxes ($200)
Sterile filtered pipette tips 20 µl, $100.00/box of 96 tips, 2 boxes ($200)
Syringes 1mL, $90.00/case of 800, 3 cases ($270)
Syringes 3mL, $90.00/case of 800, 3 cases ($270)
Syringes 5mL, $90.00/case of 800, 3 cases ($270)
Sharps containers, $43.00/case of 18, 3 cases ($108)
Trizol, $318/200mL bottle, 10 bottles ($3,180)
Viral transport media (pre-aliquotted), $2,000.00/1000mL, 1 unit ($2,000)
fine-tipped (aluminum shaft) sterile swabs, $415.00/case of 500, 5 cases ($2,075)
Puritan 6' polyester sterile swabs, $184.00/case of 1000, 3 cases ($460)
Cotton balls (med), $54.47/unit, 10 units ($545)
BD alcohol swabs, $29.10/unit, 12 units ($349)

Clean-Up materials required for the proper disposal of biohazardous and waste materials as a result of sampling efforts include autoclave/biohazard bags and antiseptic towelettes.
Y4:
Autoclave/biohazard bags large (31x38in), $134.00/case of 200, 2 cases ($268)
Autoclave/biohazard bags (19x23in), $55.00/case of 200, 3 cases ($165)
Antiseptic towelettes (Benzalkonium chloride), $63.50/case of 1000, 2 cases ($127)

From: "Kevin Olival" <olival@ecohealthalliance.org>
Sent: 05/07/2018 10:33:37 AM (-07:00)
To: "Peter Daszak" <daszak@ecohealthalliance.org>; "Jonna Mazet" <jkmazet@ucdavis.edu>; "Christine Kreuder Johnson" <ckjohnson@ucdavis.edu>; "David McIver" <dmciver@metabiota.com>; "Lindsey Shields" <ShieldsL@si.edu>; "Suzan Murray" <MurrayS@si.edu>; "Olson, Sarah" REDACTED
Cc: "Anna Willoughby" <willoughby@ecohealthalliance.org>; "Alison Andre" <andre@ecohealthalliance.org>; REDACTED; "Churchill, Carolina" REDACTED
Subject: Notes from last P2-wide M&A call, with plan for IMPACT communication/progress
Attachments: Intervention Modeling Projects ACross Teams (IMPACT) 11 April 2018.xlsx, , M&A project-wide, April 25, 2018.docx,

Dear all,

Please find attached notes from our last P2-wide M&A call. Sorry for the delay. Please let me know if you have anything to add or revise, especially in regards to the overall communication plan on IMPACT projects.

Also attaching the latest post-Napa list of IMPACT projects and point people here. Same version that Peter sent around on April 11th.

Next meeting is scheduled for: May 31st, Thursday 1 pm PST/4 pm EST. We'll follow up with call-in details closer to the date.

Cheers,
Kevin

M&A project-wide, April 25, 2018
PD, KJO, JM, CKJ, LS, DM
Meeting notes

IMPACT Projects

- *Latest version of IMAPCT project list is April 11, 2018 – version Peter sent around post-Napa. Attached again here.*
- Communication plan for IMPACTs:
    a. The "Point Person" – global lead who will keep project moving forward and lead on communication – for each project should be in contact with "Collaborators" – other global leads who have expressed an interest in helping with project and analysis – ASAP to: 1) define the analysis plan, 2) determine data sets needed (P2 data, global open source, or additional field data); and narrow down to a relevant subset of countries to focus on (ideally 2-3 countries for each IMPACT project).
        i. Collaborators on each project should also feel free to contact the Point Person during this period, to give additional ideas on the analysis plan (two way communication).
    b. Point Person will next reach out to regional leads and consortium partners (global contacts responsible for countries, not in country staff) to assess feasibility of inclusion of a country
    c. After given the okay by regional leads, Point Person should reach out to in-country teams by May 31st
        i. Point Person and collaborators should communicate with other projects within their topic area to reduce overlap especially in regards to any additional data collection needed (e.g. Both bat-hunting projects 6 & 7)
- Can use the P2-wide M&A call to report out on IMPACT progress
- Project 5 will be deleted and integrated in all projects
- Project 14 will be overseen by Tracey but likely to be run in China by Zhengli

Next Meeting is scheduled for: May 31st, Thursday 1 pm PST/4 pm EST

We will be in communication before then in regard to IMPACT projects and setting up action items and timelines

Produced in Native Format

From: REDACTED on behalf of "Jonna Mazet" <jkmazet@ucdavis.edu>
Sent: 05/22/2018 7:47:46 PM (-07:00)
To: "Beth Edison" <bedison@metabiota.com>
Cc: "PREDICT-outbreak" <predict-outbreak@ucdavis.edu>; "Brian Bird" <bhbird@ucdavis.edu>; "David Wolking" <djwolking@ucdavis.edu>; "Karen Saylors" <ksaylors@metabiota.com>; "matthew lebreton" <REDACTED>
Subject: Re: Updated supply request from MOH for Ebola Outbreak

Thanks, Beth,
I sent on to USAID/DC for guidance,
Jonna

On Tue, May 22, 2018 at 10:23 AM, Beth Edison <bedison@metabiota.com> wrote:

Hi Jonna,

We've received an updated list of requested supplies from the MOH in DRC (Attached: yellow items are available locally, red items will be provided by other partners). The local mission is working on expediting customs clearance but we don't have an update on that yet.

MOH has asked PREDICT to advise them which items on this list we can provide.
The approximate total cost for all remaining supplies will be 150k (not including IDC) and it will take 2-3 weeks to get them there once we receive notice of customs clearance.

We will need to advise MOH if we have the budget to provide everything on the list. Do you have any guidance on that?

Also, there is the option of requesting special permission from USAID to pay customs and duty fees. This would increase the cost but could potentially get the items there faster. We're looking into how much that would add to the total and whether that would actually speed things up.

Thank you,
Beth

From: predict-request@ucdavis.edu on behalf of "Elizabeth Leasure" <ealeasure@ucdavis.edu>
Sent: 06/08/2018 8:13:39 AM (-07:00)
To: "Andrew Clements" <aclements@usaid.gov>; "David John Wolking" <djwolking@ucdavis.edu>
Cc: "Alisa Pereira Emerging Threats Division" <apereira@usaid.gov>; "Amalhin Shek" <ashek@usaid.gov>; "Cara J. Chrisman" <cchrisman@usaid.gov>; "PREDICTMGT" <predictmgt@usaid.gov>; "predict@ucdavis.edu" <predict@ucdavis.edu>
Subject: RE: [predict] Re: Y5 workplan and budget guidance

Thanks, Andrew! We've added this as an agenda item for our next SMT call (next Tuesday), so hopefully you will have some information to share by that time.

Liz

Elizabeth Leasure
Financial Operations Manager
One Health Institute
REDACTED *(cell)*
530-754-9034 (office)
Skype: ealeasure

From: predict-request@ucdavis.edu <predict-request@ucdavis.edu> **On Behalf Of** Andrew Clements
Sent: Friday, June 8, 2018 5:26 AM
To: David John Wolking <djwolking@ucdavis.edu>
Cc: Alisa Pereira Emerging Threats Division <apereira@usaid.gov>; Amalhin Shek <ashek@usaid.gov>; Cara J. Chrisman <cchrisman@usaid.gov>; PREDICTMGT <predictmgt@usaid.gov>; predict@ucdavis.edu
Subject: [predict] Re: Y5 workplan and budget guidance

Hi David,

We're having an internal discussion to make sure we send all partners the same, consistent guidance on year 5 work plans.

Stay tuned....

Andrew

Andrew P. Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

On Jun 1, 2018, at 9:31 PM, David J Wolking <djwolking@ucdavis.edu> wrote:

> Hi Andrew, Alisa, and team,
>
> We are preparing for next year's workplans and as usual need to check-in on expectations and process. Over the years we have seen quite a bit of evolution on these workplans, especially for GHSA countries, so before we get started with templates, timelines, and information calls with our consortium partners we are requesting your guidance on how to proceed.

We are certainly aware of the challenging budget situation given recent discussions on our senior management team, but with respect to the actual workplans and technical content from global to country level, so far the only messages received have been from EPT-2 partner calls with reminders to engage USAID missions in the process.

We need to get started on the Y5 process relatively quickly so we can develop plans and work to align them with budgets, so would appreciate your feedback as soon as possible.

Thanks in advance and enjoy the weekend,

David

From: "Jonna Mazet" <jkmazet@ucdavis.edu>
Sent: 02/05/2019 12:32:54 PM (-08:00)
To: "AOTR/Grant Manager Andrew Clements" <AClements@usaid.gov>; "Alisa Pereira" <apereira@usaid.gov>; "Cara Chrisman" <cchrisman@usaid.gov>
Cc: "Predict inbox" <predict@ucdavis.edu>
Subject: Fwd: FW: [predict] Re: extension
Attachments: PREDICT-2 extension (starting October 2019) JM.docx

Hi,
Thanks for the updated and nicely consolidated plan draft. Here's my first pass at just some tightening up of language relative to what I believe we can deliver.
See what you think. I will also pass it through EB tomorrow and get back to you if we have any further comments.
Have a good night,
Jonna

---------- Forwarded message ---------
From: **Elizabeth Leasure** <eealeasure@ucdavis.edu>
Date: Tue, Feb 5, 2019 at 8:49 AM
Subject: FW: [predict] Re: extension
To: Jonna Mazet <jkmazet@ucdavis.edu>, David John Wolking <djwolking@ucdavis.edu>

Elizabeth Leasure

Financial Operations Manager

One Health Institute

REDACTED *(cell)*

530-754-9034 (office)

Skype: ealeasure

From: predict-request@ucdavis.edu <predict-request@ucdavis.edu> **On Behalf Of** Andrew Clements
Sent: Monday, February 4, 2019 1:33 PM
To: Jonna Mazet <jkmazet@ucdavis.edu>
Cc: Alisa Pereira <apereira@usaid.gov>; predict Sympa List <predict@ucdavis.edu>; Cara Chrisman <cchrisman@usaid.gov>
Subject: [predict] Re: extension

Hi Jonna,

We've had some additional discussion within our management team and came up with the attached outline to try to offer a succinct description of what it is we're looking for out of the extension.

Let us know what you think, including if it is achievable within the budget and time frame.

Thanks!

Andrew

Andrew Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health

U.S. Agency for International Development

Mobile phone: 1-571-345-4253
E-mail: aclements@usaid.gov

For more information on USAID's Emerging Pandemic Threats program, see: http://www.usaid.gov/ept2

On Wed, Jan 30, 2019 at 2:13 PM Jonna Mazet <jkmazet@ucdavis.edu> wrote:

> Good morning,
>
> I have prepared a revision based on my conversation with Alisa and Cara. Please see attached document. A draft budget for year 6 will follow later today.
>
> Look forward to your thoughts and to making any further revisions you recommend.
>
> Have a nice day/afternoon,
>
> Jonna
>
> On Fri, Jan 11, 2019 at 4:27 PM Jonna Mazet <jkmazet@ucdavis.edu> wrote:
>
>> Dear Andrew & Alisa,
>>
>> We discussed that we would have an extension plan to you today, but I know that it is not a great time to discuss. In any case, I thought we'd provide you with our draft plan so that it is in your inbox when the government opens. Alisa, if you think we should move forward with discussions in the interim, that's also

fine, just let me know. We're working to get the budget down to fit into the remaining ceiling, but we will need to go to the full amount if we are to achieve the objectives as laid out in this plan. We have a working Year 6 spreadsheet that we can share once we know that we are on track with the goals you envisioned.

Have a nice weekend in any case,

Jonna

On Wed, Dec 19, 2018 at 3:09 AM Andrew Clements <aclements@usaid.gov> wrote:

Sounds good. Okay with a tailored approach based on country circumstances.

After New Year is fine. How about Jan 11? We will likely need to provide a budget estimate for OAA so we'll need to know level of activities and number of countries. Timing-wise, I would like to add funding for the extension and modification of the end date done at the same time we obligate the rest of the year 5 funding so there's no funding gap. Not sure if that will happen, but if so we need to have all the pieces ready to go by February(ish).

Andrew Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health

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E-mail: aclements@usaid.gov

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On Tue, Dec 18, 2018 at 10:43 PM Jonna Mazet <jkmazet@ucdavis.edu> wrote:

Hi,

I don't remember us discussing deadlines. Happy to comply with whatever deadlines work on your end (after the holidays). We are tentatively working on the premise to try to include all GHSA Phase I countries (plus Ghana & DRC) as the baseline. As we discussed, some of these countries will have the capacity to participate (send &/or train 1 or more persons) in both advanced risk assessment analyses and policy elevation of One Health, especially wildlife. However, some countries may only have the potential for success for one or the other objective.

We have also begun discussing curricula and methods of training for different levels of investment that have already been made in the countries on the list. So while we are happy to give a list, we will likely have tailored approaches that differ amongst the countries engaged that align also with the budget available.

Sound okay?

Please advise if you have/need a deadline,

Jonna

On Tue, Dec 18, 2018 at 8:05 AM Andrew Clements <aclements@usaid.gov> wrote:

> when we met with Cara in NYC, I didn't write down the due date for your submitting the country list, etc. it might have been early january, but could be wrong.
>
> thanks
>
> Andrew Clements, Ph.D.
> Senior Scientific Advisor
> Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
>
> U.S. Agency for International Development
>
> Mobile phone: 1-571-345-4253
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>
> *For more information on USAID's Emerging Pandemic Threats program, see:* http://www.usaid.gov/ept2

PREDICT-2 extension (starting October 2019)

Goal: move towards greater sustainability for wildlife surveillance in 14 countries in Africa and Asia

Objectives:

- Include wildlife in national risk assessments for zoonotic disease threats
- Develop prioritized national strategic plans for conducting surveillance of wildlife and people (where appropriate) for zoonotic diseases[1]
- Maintain in-country data and biological samples collected during PREDICT-1 and/or PREDICT-2

Activities:

- Provide training and other support to countries to conduct risk assessments/mapping using PREDICT-2 (and other) data and modeling for zoonotic diseases from wildlife[2]
- Based on risk assessment/mapping results, provide support to countries to develop a prioritized national plan for surveillance of zoonotic diseases that include wildlife (links to national livestock and human surveillance plans as appropriate, if they exist)
- Identify and set up long-term mechanism(s) in focus countries to maintain data and biological samples collected during PREDICT-1 and/or PREDICT-2

Outputs and outcomes:

- Each country has staff who are trained in conducting risk assessments for zoonotic diseases from wildlife
  (indicator: percent of focus countries with capacity to conduct risk assessments/mapping for zoonotic wildlife diseases)
- Each focus country has a completed risk assessment/map for zoonotic diseases, including risk from wildlife spillover (indicator: percent of focus countries completing risk assessments/mapping for zoonotic diseases from wildlife)
- Each focus country has a drafted prioritized national plan for zoonotic disease surveillance from wildlife
  (indicator: percent of focus countries with a drafted prioritized national plan for zoonotic disease surveillance that includes wildlife)
- Each focus country has a long-term plan for maintaining its data and biological samples collected under PREDICT-1 and/or PREDICT-2
  (indicator: percent of focus countries with a data and sample management plan)

---

[1] Operationalization of these plans to be supported by other partners (TBD) under USAID's Next Generation portfolio (2019-2024).

[2] Link to Zoonotic Disease Prioritization process.

From: "Andrew Clements" <aclements@usaid.gov>
Sent: 02/06/2019 2:09:57 PM (-08:00)
To: "Jonna Mazet" <jkmazet@ucdavis.edu>
Cc: "Alisa Pereira" <apereira@usaid.gov>; "Cara Chrisman" <cchrisman@usaid.gov>; "Predict inbox" <predict@ucdavis.edu>
Subject: Re: FW: [predict] Re: extension
Attachments: PREDICT-2 extension (starting October 2019) JM__AC.docx

Hi Jonna,

Thanks for your feedback.

See attached for some comments in response to some of your edits as well as some new thoughts/requests to continue building it out. As noted in one comment, we'd like to schedule a call with you later this week or early next week to get an update on the status of countries developing plans to manage their samples and data.

Andrew

Andrew Clements, Ph.D.
Senior Scientific Advisor
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Date: Tue, Feb 5, 2019 at 8:49 AM
Subject: FW: [predict] Re: extension
To: Jonna Mazet <jkmazet@ucdavis.edu>, David John Wolking <djwolking@ucdavis.edu>

Elizabeth Leasure

Financial Operations Manager

One Health Institute

REDACTED *(cell)*

530-754-9034 (office)

Skype: ealeasure

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Andrew Clements, Ph.D.
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Please advise if you have/need a deadline,

Jonna

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thanks

Andrew Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health

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E-mail: aclements@usaid.gov

For more information on USAID's Emerging Pandemic Threats program, see: http://www.usaid.gov/ept2

PREDICT-2 extension (starting October 2019)

Goal: move towards greater sustainability for wildlife surveillance in 14 countries in Africa and Asia

Objectives:

- Include priority microbes from wildlife in national risk assessments for zoonotic disease threats
- Develop prioritized national strategic plans for conducting surveillance of wildlife and high-risk people (where appropriate) for zoonotic diseases[1]
- Maintain in-country data and biological samples collected during PREDICT-1 and/or PREDICT-2

Activities:

- 1. Provide training and other support to countries to analyze their surveillance and modeling data (from PREDICT-2 and others) for zoonotic diseases from wildlife and conduct risk assessments/mapping[2]

  Time frame:

  Data analysis -- [Month, year] to [Month, year] (if not already done by Sep 2019)

  Risk assessment/mapping -- [Month, year] to [Month, year]

- 2. Based on risk assessment/mapping results, provide support to countries to develop a prioritized national plan for surveillance of zoonotic diseases that include wildlife (links to national livestock and human surveillance plans as appropriate, if they exist)

  Time frame:

  - Develop prioritized national surveillance plan -- [Month, year] to [Month, year]

- 3. If not already accomplished by September 30, 2019[3], identify and set up long-term non-PREDICT mechanism(s) in focus countries to maintain data and biological samples collected during PREDICT-1 and/or PREDICT-2

  Time frame:

  Identify mechanisms -- [Month, year] to [Month, year]

  Set-up mechanisms -- [Month, year] to [Month, year]

[1] Operationalization of these plans to be supported by other partners (TBD) under USAID's Next Generation portfolio (2019-2024).
[2] Link to Zoonotic Disease Prioritization process.
[3] PREDICT-2 year 5 work plans include developing plans for countries to manage their data and samples.

<u>Outputs and outcomes</u>:

- Each country has staff who are trained in conducting risk assessments for zoonotic diseases from wildlife
  (indicator: percent of focus countries with capacity to conduct risk assessments/mapping for zoonotic wildlife diseases)
- Each focus country has a completed risk assessment/map for zoonotic diseases, including risk from wildlife spillover (indicator: percent of focus countries completing risk assessments/mapping for zoonotic diseases from wildlife)
- Each focus country has a drafted prioritized national plan for zoonotic disease surveillance from wildlife
  (indicator: percent of focus countries with a drafted prioritized national plan for zoonotic disease surveillance that includes wildlife)
- Each focus country has a long-term plan for maintaining its data and biological samples collected under PREDICT-1 and/or PREDICT-2
  (indicator: percent of focus countries with a data and sample management plan)

<u>Budget</u>:

Estimate for activity 1 = $

Estimated for activity 2 = $

Estimate for activity 3 = $

Total = $

From: "Jonna Mazet" <jkmazet@ucdavis.edu>
Sent: 02/06/2019 5:14:51 PM (-08:00)
To: "Andrew Clements" <aclements@usaid.gov>
Cc: "Alisa Pereira" <apereira@usaid.gov>; "Cara Chrisman" <cchrisman@usaid.gov>; "Predict inbox" <predict@ucdavis.edu>
Subject: Re: FW: [predict] Re: extension

Thanks, Andrew.
Your revision looks really good to me & is fleshing-out nicely.

My only major comment is around the breakdown of activities and timelines under Activity 1, which read:
"Data analysis -- [Month, year] to [Month, year] (if not already done by Sep 2019)
Risk assessment/mapping -- [Month, year] to [Month, year]"

I think this would be better structured as “Training” on the first line and then “Data analyses/risk assessment/mapping”, as the data analyses use risk assessment methods & both the risk assessment & the mapping are products of the analyses. Does that make sense? In any case, I wouldn't know how to separate out the steps, as written, especially with timelines. Training certainly does need to come first and could have a separate timeline. The only issue I have with setting timing around these is that different individuals will pick-up the material and assimilate it for use at different speeds, so while the training for this phase would all officially start in October (if not before, depending on country progress on rest of P-2 objectives & flow of funding for this year), the beginning of the next phase will be country-dependent. That said, we could ball-park the start date of the second piece.

If you agree with a change as described above, I can add approximate timelines to the spots indicated on the draft and send it back.

Philosophical question:
The budget I sent previously does not break down costs by these activities because there is no clear break-down along those lines, except possibly apportioning by months according to the timelines that I would insert in the next draft. Again, the reason for that is that we would be training and then supporting the budgeted in-country staff to accomplish the three activities along a continuum of progress of their training & analytical skills and according to how long things take to set up mechanisms (third activity) in each country. So the level of effort for the three activities would definitely differ by country and is extremely difficult to predict until we start with the trainee. Hence the more predictable way to go about budgeting this phase is to plan for the support of the in-country staff and their mentors for the whole period with travel, as I provided. If you want me to just apportion time to those activities roughly across anticipated average timelines, I could do that, but it won't likely reflect reality. Also, if someone came along and said cut activity 3, there would not be any associated cost savings because the staff person would still be working on activity 2 in the same timeframe.

Let me know how you'd like me to proceed & thanks for your ongoing support for these important activities,
Jonna

On Wed, Feb 6, 2019 at 2:10 PM Andrew Clements <aclements@usaid.gov> wrote:

Hi Jonna,

Thanks for your feedback.

See attached for some comments in response to some of your edits as well as some new thoughts/requests to continue building it out. As noted in one comment, we'd like to schedule a call with you later this week or early next week to get an update on the status of countries developing plans to manage their samples and data.

Andrew

Andrew Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
E-mail: aclements@usaid.gov

For more information on USAID's Emerging Pandemic Threats program, see: http://www.usaid.gov/ept2

On Tue, Feb 5, 2019 at 9:35 PM Jonna Mazet <jkmazet@ucdavis.edu> wrote:

Hi,

Thanks for the updated and nicely consolidated plan draft. Here's my first pass at just some tightening up of language relative to what I believe we can deliver.

See what you think. I will also pass it through EB tomorrow and get back to you if we have any further comments.

Have a good night,

Jonna

---------- Forwarded message ---------
From: **Elizabeth Leasure** <ealeasure@ucdavis.edu>
Date: Tue, Feb 5, 2019 at 8:49 AM
Subject: FW: [predict] Re: extension
To: Jonna Mazet <jkmazet@ucdavis.edu>, David John Wolking <djwolking@ucdavis.edu>

Elizabeth Leasure

Financial Operations Manager

One Health Institute

[redacted] *(cell)*

530-754-9034 (office)

Skype: ealeasure

From: predict-request@ucdavis.edu <predict-request@ucdavis.edu> **On Behalf Of** Andrew Clements
Sent: Monday, February 4, 2019 1:33 PM
To: Jonna Mazet <jkmazet@ucdavis.edu>
Cc: Alisa Pereira <apereira@usaid.gov>; predict Sympa List <predict@ucdavis.edu>; Cara Chrisman <cchrisman@usaid.gov>
Subject: [predict] Re: extension

Hi Jonna,

We've had some additional discussion within our management team and came up with the attached outline to try to offer a succinct description of what it is we're looking for out of the extension.

Let us know what you think, including if it is achievable within the budget and time frame.

Thanks!

Andrew

Andrew Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health

U.S. Agency for International Development

Mobile phone: 1-571-345-4253
E-mail: aclements@usaid.gov

For more information on USAID's Emerging Pandemic Threats program, see: http://www.usaid.gov/ept2

On Wed, Jan 30, 2019 at 2:13 PM Jonna Mazet <jkmazet@ucdavis.edu> wrote:

Good morning,

I have prepared a revision based on my conversation with Alisa and Cara. Please see attached document. A draft budget for year 6 will follow later today.

Look forward to your thoughts and to making any further revisions you recommend.

Have a nice day/afternoon,

Jonna

On Fri, Jan 11, 2019 at 4:27 PM Jonna Mazet <jkmazet@ucdavis.edu> wrote:

Dear Andrew & Alisa,

We discussed that we would have an extension plan to you today, but I know that it is not a great time to discuss. In any case, I thought we'd provide you with our draft plan so that it is in your inbox when the government opens. Alisa, if you think we should move forward with discussions in the interim, that's also fine, just let me know. We're working to get the budget down to fit into the remaining ceiling, but we will need to go to the full amount if we are to achieve the objectives as laid out in this plan. We have a working Year 6 spreadsheet that we can share once we know that we are on track with the goals you envisioned.

Have a nice weekend in any case,

Jonna

On Wed, Dec 19, 2018 at 3:09 AM Andrew Clements <aclements@usaid.gov> wrote:

Sounds good. Okay with a tailored approach based on country circumstances.

After New Year is fine. How about Jan 11? We will likely need to provide a budget estimate for OAA so we'll need to know level of activities and number of countries. Timing-wise, I would like to add funding for the extension and modification of the end date done at the same time we obligate the rest of the year 5 funding so there's no funding gap. Not sure if that will happen, but if so we need to have all the pieces ready to go by February(ish).

Andrew Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health

U.S. Agency for International Development

Mobile phone: 1-571-345-4253
E-mail: aclements@usaid.gov

For more information on USAID's Emerging Pandemic Threats program, see: http://www.usaid.gov/ept2

On Tue, Dec 18, 2018 at 10:43 PM Jonna Mazet <jkmazet@ucdavis.edu> wrote:

Hi,

I don't remember us discussing deadlines. Happy to comply with whatever deadlines work on your end (after the holidays). We are tentatively working on the premise to try to include all GHSA Phase I countries (plus Ghana & DRC) as the baseline. As we discussed, some of these countries will have the capacity to participate (send &/or train 1 or more persons) in both advanced risk assessment analyses and policy elevation of One Health, especially wildlife. However, some countries may only have the potential for success for one or the other objective.

We have also begun discussing curricula and methods of training for different levels of investment that have already been made in the countries on the list. So while we are happy to give a list, we will likely have tailored approaches that differ amongst the countries engaged that align also with the budget available.

Sound okay?

Please advise if you have/need a deadline,

Jonna

On Tue, Dec 18, 2018 at 8:05 AM Andrew Clements <aclements@usaid.gov> wrote:

> when we met with Cara in NYC, I didn't write down the due date for your submitting the country list, etc. it might have been early january, but could be wrong.
>
> thanks
>
> Andrew Clements, Ph.D.
> Senior Scientific Advisor
> Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
>
> U.S. Agency for International Development
>
> Mobile phone: 1-571-345-4253
> E-mail: aclements@usaid.gov
>
> *For more information on USAID's Emerging Pandemic Threats program, see:* http://www.usaid.gov/ept2

From: "Darsema Gulima Huluka" <dhuluka@hrh2030program.org>
Sent: 03/06/2019 1:08:55 AM (-08:00)
To: "Faith Bartz Tarr" <fbartz@usaid.gov>; "Lisa Kramer" <lkramer@usaid.gov>; "Woutrina A Smith" <wasmith@ucdavis.edu>; "hellen Amuguni" <Janetrix.Amuguni@tufts.edu>; "Innocent Rwego" REDACTED "Diafuka Saila-Ngita" <diafuka.saila_ngita@tufts.edu>; "David Mutonga" <david.mutonga@thepalladiumgroup.com>; "Katey Pelican" <pelicank@umn.edu>; "Nigatu kebede" REDACTED "Lindsay Parish" <lparish@usaid.gov>; "Andrea Long-Wagar" <alongwagar@usaid.gov>; "Alisa Pereira" <apereira@usaid.gov>; "Ashna Kibria" <akibria@usaid.gov>; "Andrew Clements" <aclements@usaid.gov>; "Jonna Mazet" <jkmazet@ucdavis.edu>; "Tzipori, Saul" <saul.tzipori@tufts.edu>; "Makonnen, Yilma (FAORNE)" REDACTED "Marilyn Crane" <mcrane@usaid.gov>; "Innocent Rwego" <irwego@umn.edu>; "Andrew Kitua" REDACTED 'Ricardo Echalar" <rechalar@usaid.gov>; "Jeff Bender" <bende002@umn.edu>; "Susan Scribner" REDACTED ; "Mandy Paust" <apaust@usaid.gov>; "Yirgalem Gebremeskel" <ygebremeskel@usaid.gov>; "Woldtsadique, Feleseta (FAOET)" REDACTED "Awoke, Wondwosen (FAOET)" REDACTED VantKlooster, Gijs (FAOET)" REDACTED ; "Jennifer K Lane" <jklane@ucdavis.edu>; "Simon Heliso" <Simonh@jhuccpeth.org>; "Guda Alemayehu" <galemayehu@usaid.gov>; "Anton Schneider" <aschneider@usaid.gov>; "Tegegne Shiferaw" <tegegnes@jhuccpeth.org>; "Khadijah Alibhai" <kalibhai@brynmawr.edu>; "Darsema Gulima" REDACTED "filimonab@crdaethiopia.org" <filimonab@crdaethiopia.org>; "Legesse Bezabih" REDACTED "Muluken Alemu" REDACTED ; "Mohan Joshi" <mjoshi@msh.org>; "Mekonnen,Negussu" <nmekonnen@msh.org>; "Johnson, Denise" <Denise.Johnson@icf.com>; "Benita Izere" <Benita.Izere@icf.com>; "Asnakew Yeshiwondim" <ayeshiwondim@path.org>; "Maria Busquets" <mbusquets@usaid.gov>
Cc: "hrh one health pmu" <hrhonehealthpmu@chemonics.com>; "Grace Tran" <gtran@chemonics.com>; "Mariam Reda" <mreda@chemonics.com>
Subject: RE: Ethiopia GHSA biweekly update, Jan. 25
Attachments: HRH2030 OH-GHSA_Ethiopia_BiWeekly update, Febr 6,19_.docx

Dear Dr Faith,
Kindest greetings.
Please find attached the HRH2030 One Health GHSA biweekly updates.
With best regards,
Darsema Gulima Huluka
Multisectoral Health Security Advisor
Human Resources for Health in 2030 One Health Project
United States Agency for International Development (USAID)
Email: dhuluka@hrh2030program.org
REDACTED
Cellphone: REDACTED
Skype: REDACTED
REDACTED
From: Faith Bartz Tarr <fbartz@usaid.gov>
Sent: Monday, March 4, 2019 8:30 AM
To: Lisa Kramer <lkramer@usaid.gov>; Woutrina A Smith <wasmith@ucdavis.edu>; hellen Amuguni <Janetrix.Amuguni@tufts.edu>; Innocent Rwego REDACTED Diafuka Saila-Ngita <diafuka.saila_ngita@tufts.edu>; David Mutonga <david.mutonga@thepalladiumgroup.com>; Katey Pelican <pelicank@umn.edu>; Nigatu kebede REDACTED Lindsay Parish <lparish@usaid.gov>; Andrea Long-Wagar <alongwagar@usaid.gov>; Alisa Pereira <apereira@usaid.gov>; Ashna Kibria <akibria@usaid.gov>; Andrew Clements <aclements@usaid.gov>; Jonna Mazet <jkmazet@ucdavis.edu>; Tzipori, Saul <saul.tzipori@tufts.edu>; Makonnen, Yilma (FAORNE) REDACTED Marilyn Crane <mcrane@usaid.gov>; Innocent Rwego <irwego@umn.edu>; Andrew Kitua REDACTED Ricardo Echalar <rechalar@usaid.gov>; Jeff Bender <bende002@umn.edu>; Susan Scribner REDACTED ; Mandy Paust <apaust@usaid.gov>; Yirgalem Gebremeskel <ygebremeskel@usaid.gov>; Woldtsadique, Feleseta (FAOET) REDACTED ; Awoke,

Wondwosen (FAOET) [redacted] VantKlooster, Gijs (FAOET) [redacted] Jennifer K Lane <jklane@ucdavis.edu>; Simon Heliso <Simonh@jhuccpeth.org>; Guda Alemayehu <galemayehu@usaid.gov>; Anton Schneider <aschneider@usaid.gov>; Tegegne Shiferaw <tegegnes@jhuccpeth.org>; Khadijah Alibhai <kalibhai@brynmawr.edu>; Darsema Gulima [redacted] Darsema Gulima Huluka <dhuluka@hrh2030program.org>; [redacted] filimonab@crdaethiopia.org; Legesse Bezabih [redacted]; Muluken Alemu [redacted]>; Mohan Joshi <mjoshi@msh.org>; Mekonnen,Negussu <nmekonnen@msh.org>; Johnson, Denise <Denise.Johnson@icf.com>; Benita Izere <Benita.Izere@icf.com>; Asnakew Yeshiwondim <ayeshiwondim@path.org>; Maria Busquets <mbusquets@usaid.gov>
Subject: Re: Ethiopia GHSA biweekly update, Jan. 25

Dear GHSA Ethiopia colleagues,

It's time for the biweekly update on GHSA activities in Ethiopia. This should cover activities from Feb. 23 - March 8, 2019. Please use the attached template, and send by COB Wed. March 6.

Thank you all as always, and wishing you well.
Regards,

Faith Bartz Tarr, Ph.D.

USAID Ethiopia

Acting Global Health Security Advisor
AAAS Science and Technology Policy Fellow & Agriculture Officer
Office of Economic Growth and Transformation

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Twitter: @USAIDEthiopia

Ethiopia GHSA Implementation Bi-Weekly Updates

USAID Implementing Partners

Date Submitted	06 February 2019
Project	HRH2030 One Health

I. **<u>Highlighted Updates</u>: Please list (maximum five) major updates on activity implementation in Ethiopia.**

FORMAT: Insert 1 sentence summary of update (bold). Insert 1-3 sentences of additional information.

1. **Provide technical support to the EPT TWG in the development of detail Work Plan and indicative budget for the Ethiopian multisectoral HPAI PRP**

HRH2030 OH MHSA provided Technical support to the EPT Technical Working Group in the development of detail Workplan and indicative budget for the multisectoral Highly Pathogenic Avian Influenza (HPAI) Preparedness and response Plan (PRP) reviewed last week with the support of HRH2030. The three days workshop was held from 21st to 23rd February 2019 in Bishoftu, Ethiopia.

The objectives of the workshop were:

- Develop a draft detail workplan for the multisectoral HPAI Preparedness and response Plan
- Develop an indicative budget for the implementation of the multisectoral HPAI Preparedness and Response Plan
- Finalize the contact list of the multisectoral HPAI Preparedness and Response Plan to ensure an effective and sustainable multisectoral collaboration mechanism (MCM) in the implementation of the multisectoral HPAI Preparedness and Response Plan
- Outline and agree on the follow up action for the finalization and launching of the multisectoral HPAI Preparedness Response Plan.

2. **Participation and providing technical support to the Brucellosis Evaluation and National Strategy Development Workshop**

From February 27-28, 2019, MHSA of HRH2030 One Health participated and contributed to the Brucellosis Evaluation and National Strategy Development Workshop organized by the national Brucellosis Technical Working Group (BrTWG). The two days' workshop was held in Bishoftu, Ethiopia in the presence of the BrTWG members, line ministries representatives and development partners including CDC Atlanta, CDC Ethiopia, FAO, JHU, OSU-GOHi, HRH2030 One Health, relevant directorates of Ethiopian Public Health Institute and National Animal Health Diagnostic and Investigation Center (NAHDIC), National Veterinary Institute, Jimma and Addis Ababa Universities.

The Workshop objectives were:

1. Discuss general Brucellosis background and evaluate the current capacity of Brucellosis Prevention and Control activities in the country
2. Introduce the FAO "Stepwise Approach for the Progressive Control of Brucellosis" Guidance and the Staged Tool for the Elimination of Brucellosis (STEB)
3. Conduct a STEB assessment
4. Identify priority activities to include in Ethiopia's National Brucellosis Prevention, Control and Elimination Strategy

The STEB is a Microsoft Excel-based tool that is being piloted for countries to use as a staging tool to assess their status, develop activities, and monitor progress towards brucellosis prevention, control, and elimination. The most effective way to alleviate the burden of brucellosis within a country is known to be reducing the prevalence in livestock. Therefore, the STEB primarily focuses on reaching elimination through controlling the disease in livestock.

Purpose of the STEB

This tool has been developed to assist countries in evaluating their current capacity to control and eliminate Brucellosis. By Identifying gaps in capacity, the STEB can be used as a self-assessment tool to assist countries in developing activities that utilize o OH approach to Br control and prevention. This tool is intended to be used in combination with the guidance document, "a Stepwise approach for the progressive control of Br in Livestock". When used in conjunction with this document, the STEB provides practical guidance on how to implement Br control activities, and how to monitor and advance progress toward elimination in livestock.

The STEB utilizes a One Health approach to brucellosis control and elimination. Therefore, the tool is intended to be completed with members of all relevant sectors (e.g., agriculture/livestock health, human/public health, wildlife/environment, One Health) present and participating in responses. As such, there are four sections with questions: Ministry of Agriculture (questions relating to brucellosis in livestock) Ministry of Health (questions relating to brucellosis in people) and All/One Health (questions relating to collaborative or joint activities) and Wildlife (questions relating to brucellosis in wild animal populations).

Approach of the workshop

- In the workshop, various presentations were delivered and discussed up on including:
- Brucellosis Technical Working Group Updates

- Overview of the FAO's "Stepwise Approach to Brucellosis Control"
- Brucellosis overview in humans and animals with emphasis in Ethiopia
- Introduction in to the World Style Café Activity followed by exercise through which identified the activities of the prevention, control and elimination strategy of Ethiopia

Following the presentations, the participants split in to groups and discussed on the findings and gaps identified and should be given a due attention in the development of the national Brucellosis prevention, control and elimination strategy.

After the self-evaluation and identification of gaps, priority multisectoral and sector specific activities of the National Brucellosis prevention, control and elimination Strategy were identified. Finally, the next steps (way forwards) were pointed out and agreed up on for multisectoral efforts and joint actions.

Next steps

- Conduct synopsis of the outcome of the workshop
- Prepare and share the preliminary workshop report (by March 15, 2019)
- Establish timeline for activities
- Share the outcomes with the NOHSC during its next meeting
- Identify responsible individuals from all sectors to coordinate and implement the activities
- Prepare and share a progress monitoring guide
- Summaries all available reports and research findings and conduct mega analysis
- Identify resources required for each of the planned activities
- Preparation of multisectoral Brucellosis prevention, control and elimination strategy (first draft-July 2019; Approval of the final by the end of 2019)

II. <u>Coordination</u>: Please describe any activities in Ethiopia that may benefit from coordination with other GHSA implementing partners or USG agencies (e.g. CDC, USDA, DTRA, etc.) and how.

Establishment and functionalization of regional OH platforms is a good opportunity collaboration and partnership with other GHSA implementing partners such as Embassy of Swiss/VSF-Suisse, DETRA and JHU CCP)

III. <u>Challenges</u>: Please describe any significant challenges related to planning or implementation of GHSA activities in Ethiopia.

The delay on the process of project workplan approval hindered to start the planned project activities implementation

IV. <u>Upcoming GHSA related TDYs</u>: Please provide the information requested below on all GHSA-related TDYs to Ethiopia for the next six weeks. Insert additional rows as necessary.

Traveler(s)	**Location (areas to be visited)**	**Dates**	**Trip Objectives**	**Trip Impact (including deliverables)** *This should also specify if/how this TDY will build host nation capacity and contributes to overarching GHS objectives*

V. Upcoming major GHSA related meetings/trainings/events for the next six weeks (if information is not captured in TDY table above)

Meeting/Training/ Event Topic	Location	Dates	Objectives	Number and type of participants
Writeshop for development of multisectoral RVF preparedness and response plan	Adama/ Bishoftu	25th to 30th March 2019	Draft an outline for a comprehensive multisectoral RVF PRP for Ethiopia that enables the country to effectively prevent the introduction of RVF into its territory, rapidly respond to and contain any RVF outbreak in the country.	12 subject matter specialists drawn from core ministries, academia and development partners
GHSA annual meeting	Sydney, Australia	16-22 June 2019	Experience sharing	2 (MHSA & NOHSC chair)

From: "VantKlooster, Gijs (FAOET)" <[redacted]
Sent: 03/06/2019 3:27:18 AM (-08:00)
To: "Faith Bartz Tarr" <fbartz@usaid.gov>
Cc: "Lisa Kramer" <lkramer@usaid.gov>; "Woutrina A Smith" <wasmith@ucdavis.edu>; "hellen Amuguni" <Janetrix.Amuguni@tufts.edu>; "Innocent Rwego" [redacted] "Diafuka Saila-Ngita" <diafuka.saila_ngita@tufts.edu>; "David Mutonga" <david.mutonga@thepalladiumgroup.com>; "Katey Pelican" <pelicank@umn.edu>; "Nigatu kebede" [redacted] "Lindsay Parish" <lparish@usaid.gov>; "Andrea Long-Wagar" <alongwagar@usaid.gov>; "Alisa Pereira" <apereira@usaid.gov>; "Ashna Kibria" <akibria@usaid.gov>; "Andrew Clements" <aclements@usaid.gov>; "Jonna Mazet" <jkmazet@ucdavis.edu>; "Tzipori, Saul" <saul.tzipori@tufts.edu>; "Makonnen, Yilma (AGAH)" [redacted] Marilyn Crane" <mcrane@usaid.gov>; "Innocent Rwego" <irwego@umn.edu>; "Andrew Kitua" [redacted] "Ricardo Echalar" <rechalar@usaid.gov>; "Jeff Bender" <bende002@umn.edu>; "Susan Scribner" [redacted] "Amanda Paust" <apaust@usaid.gov>; "Yirgalem Gebremeskel" <ygebremeskel@usaid.gov>; "Woldtsadique, Feleseta (FAOET)" [redacted] "Awoke, Wondwosen (FAOET)" [redacted] Jennifer K Lane" <jklane@ucdavis.edu>; "Simon Heliso" <Simonh@jhuccpeth.org>; "Guda Alemayehu" <galemayehu@usaid.gov>; "Anton Schneider" <aschneider@usaid.gov>; "Tegegne Shiferaw" <tegegnes@jhuccpeth.org>; "Khadijah Alibhai" <kalibhai@brynmawr.edu>; "Darsema Gulima" [redacted] "Darsema Gulima Huluka" <dhuluka@hrh2030program.org>; [redacted] "filimonab@crdaethiopia.org" <filimonab@crdaethiopia.org>; "Legesse Bezabih" [redacted] "Muluken Alemu" [redacted] "Mohan Joshi" <mjoshi@msh.org>; "Mekonnen,Negussu" <nmekonnen@msh.org>; "Johnson, Denise" <Denise.Johnson@icf.com>; "Benita Izere" <Benita.Izere@icf.com>; "Asnakew Yeshiwondim" <ayeshiwondim@path.org>; "Maria Busquets" <mbusquets@usaid.gov>
Subject: RE: Ethiopia GHSA biweekly update, Jan. 25
Attachments: FAO ECTAD EPT2-GHSA Ethiopia BiWeekly Update 2019-02-23 to 2019-03-08.docx

Dear Faith,

Please do find attached the bi-weekly report covering the activities from Feb. 23 - March 8, 2019.
Wishing you a nice afternoon!

Kind regards
Gijs

Gijs van 't Klooster
Head Livestock and Pastoralism Thematic Programme Food and Agriculture Organization of the United Nations (FAO)
[redacted]

Email: [redacted]
Office: [redacted]
Mobile: [redacted]

Kind regards
Gijs

From: Faith Bartz Tarr <fbartz@usaid.gov>
Sent: Monday, March 4, 2019 8:30 AM
To: Lisa Kramer <lkramer@usaid.gov>; Woutrina A Smith <wasmith@ucdavis.edu>; hellen Amuguni <Janetrix.Amuguni@tufts.edu>; Innocent Rwego [redacted] Diafuka Saila-Ngita

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Subject: Re: Ethiopia GHSA biweekly update, Jan. 25

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Thank you all as always, and wishing you well.
Regards,

Faith Bartz Tarr, Ph.D.

USAID Ethiopia

Acting Global Health Security Advisor
AAAS Science and Technology Policy Fellow & Agriculture Officer
Office of Economic Growth and Transformation

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USAID.gov/ethiopia
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Twitter: @USAIDEthiopia

Ethiopia GHSA Implementation Bi-Weekly Updates
USAID Implementing Partners

Date Submitted	6 March 2019
Project	ECTAD Ethiopia

I. <u>Highlighted Updates</u>: Please list (maximum five) major updates on activity implementation in Ethiopia.

FORMAT: Insert 1 sentence summary of update (bold). Insert 1-3 sentences of additional information.

1. **Under the Zoonotic Diseases Action Package, indicator: P.4.1. Surveillance systems in place for priority zoonotic diseases/pathogens,** FAO participated and contributed to ToT training organized in collaboration with MoA on real time mobile phone reporting system. This two-day ToT training, attended by 39 people, took place at Adama from 21-22 Feb 2019. Participants were drawn from Regional veterinary service and laboratory personnel from nine regional states and two city administrations. The way forward to cascade the training to their respective areas were also discussed.
2. **Under the Zoonotic Diseases Action Package, indicator: P.4.1. Surveillance systems in place for priority zoonotic diseases/pathogens,** CDC in collaboration with FAO organized consultative workshop from 27-28 Feb 2019 in Bishoftu. The purpose of the workshop was to apply CDC-FAO developed SBET (Step-wise Brucellosis eradication Tool) to support country-level brucellosis prevention and control strategy development. A total of 19 participants mainly Brucellosis TWG members, FAO, CDC, OSU-GOHi and other partners participated in the workshop.
3. **Under the National Laboratory Systems Action Package, Indicator D.1.1. Laboratory testing for detection of priority diseases, *Activity 2. Strengthen the capacity of selected sub-national labs to conduct 3 core diagnostic tests (HPAI, brucellosis and RVF):*** Training scheduled from 4 – 8 March 2019 is underway at NAHDIC to improve the knowledge of selected sub-national labs and junior staff of NAHDIC on RVF to collect, handle, transport and/or test appropriate samples in case of suspected cases of the disease. In addition, laboratory supplies for RVF are being procured so that NAHDIC and selected regional state labs can conduct annual active surveillance in search of the virus. A total of fifteen participants i.e. nine from regional state labs (Jigjiga, Yabello, Assosa, Jinka and Dire Dewa) and 6 staff of NAHDIC are attending at the training.
4. **Under the National Laboratory Systems Action Package, Indicator D.1.2 Specimen referral and transport system, Activity 4. Training on International Air Transport Association (IATA),** Two NAHDIC staff are attending a regional training workshop on Diagnostic sample packaging and shipment in Nairobi, Kenya 5-8 March 2019. The training brought together candidates from Anglophone countries in East African countries, namely Ethiopia, Kenya, Uganda and the United Republic of Tanzania, who will acquire the necessary competencies to be able to send diagnostic specimens according to the international regulations. The objective of the training is to create at least one, preferably two valid certificate holders per country. Upon successful attendance and completion of the final examination (mark 80% or higher) the participants will receive a Certificate of the 'Training on Transport of Infectious Substance by Air'. The certificate will be valid for two years from the certification date.
5. **ASL2050:** Revised the Livestock Scenario Report and submitted to FAO HQ Publication unit for further proofreading and typesetting in preparation for publication. A manuscript entitled *"Livestock Growth, Public Health and the Environment in Ethiopia: A Quantitative Assessment"* has been prepared in collaboration with the Global

P&R Team. The manuscript presents quantitative assessment of the impact of zoonotic diseases on public health in the year 2050 using the One Health Policy Model.

6. **ASL2050:** Reviewed and provided feedback on a document "*Poultry Sector Country Review: Ethiopia*" prepared by FAO Animal Production and Health Division.

II. <u>Coordination</u>: Please describe any activities in Ethiopia that may benefit from coordination with other GHSA implementing partners or USG agencies (e.g. CDC, USDA, DTRA, etc.) and how.

- As part of the process to identify, document and share the contribution of SILAB implementation to zoonotic diseases detection and response efforts in Ethiopia, ECTAD Ethiopia had discussion with relevant NAHDIC staff on 4 March 2019.

III. <u>Challenges</u>: Please describe any significant challenges related to planning or implementation of GHSA activities in Ethiopia.

\- None -

IV. <u>Upcoming GHSA related TDYs</u>: Please provide the information requested below on all GHSA-related TDYs to Ethiopia for the next six weeks. Insert additional rows as necessary.

Traveler(s)	**Location (areas to be visited)**	**Dates**	**Trip Objectives**	**Trip Impact (including deliverables)** *This should also specify if/how this TDY will build host nation capacity and contributes to overarching GHS objectives*
Tadele Mirkena	Rome	25-28 March, 2019	To participate in ASL2050 Regional Meeting and Policy Training	Provide FAO's ASL2050 focal points with the capacity to utilize policy-related tools developed by different FAO departments and units to support ASL2050 implementation in their country of responsibility.

V. <u>Upcoming major GHSA related meetings/trainings/events</u> for the next six weeks (if information is not captured in TDY table above)

Meeting/Training/ Event Topic	**Location**	**Dates**	**Objectives**	**Number and type of participants**
Visit of Teramo consultants to sub-national labs	Assela	11 – 15 March 2019	To look into possibilities of linking them with NAHDIC using SILAB	Two staff from Teramo and one from NAHDIC

From:	Corina Grigorescu Monagin <cgmonagin@ucdavis.edu>
To:	William B. Karesh <karesh@ecohealthalliance.org>;Catherine Machalaba <machalaba@ecohealthalliance.org>
CC:	predict Sympa List <predict@ucdavis.edu>;David John Wolking <djwolking@ucdavis.edu>;Molly Turner <turner@ecohealthalliance.org>;Ava Sullivan <sullivan@ecohealthalliance.org>
Sent:	4/9/2019 10:00:07 AM
Subject:	[predict] Re: Action required: 2019 Semi-annual Report due to UCD HQ April 5, 2019

Hey there,

Just a friendly reminder to send me your M&E section when you have a chance. Any questions, let me know.

Thanks!
Corina

From: David Wolking
Date: Wednesday, March 20, 2019 at 12:00 PM
To: William Karesh , Catherine Machalaba
Cc: Corina Grigorescu Monagin , predict Sympa List , Molly Turner , Ava Sullivan
Subject: Action required: 2019 Semi-annual Report due to UCD HQ April 5, 2019

Hi there,

It's reporting season once again!

Global section:
I've attached the text from your section of the 2018 Annual Report for quick reference and to update as a template if you like. Remember the Semi-annual Report covers the 6 month period of performance (October 1, 2018-March 31, 2019), but in many cases we are showing life of project progress and achievements.

The deadline for EIDITH data entry for country teams is March 31. ***Therefore, the deadline for submission of these semi-annual report sections is April 5, 2019.*** Unlike the annual report we only have 30 days to turn this around for USAID and these now take a lot of design time. Typically the global section of these semi-annual reports is a more concise and abbreviated, so the deadline should be feasible. Here's a link to last year's semi-annual for quick reference.

Because we are now designing these report in InDesign, please provide any figures, photos, etc. as separate files with specific links in the text for ease of reference.

M&E Section:
The M&E data call is also for 6 months (October 1, 2018 – March 31, 2019). We've attached a clean workbook here for data entry. Most instructions are included on the template itself but please refer to the indicator reference sheet if you have questions (or reach out to Corina and I).

One Health (Billy/Catherine):
2B: Qualitative Indicator: Evidence of application of OH trainings and sensitization in the workforce; (country info captured in our questionnaire).
2.1b: #, list of publicly available educational, training, and/or implementation resources developed and shared (country info captured in our questionnaire and consult with Capacity Team)
3B: Qualitative Indicator: List of global, regional or country strategies under implementation (country info captured in our questionnaire).
3.2a: #, list of evidence-based informational resources developed or refined (consult with M&A, Lab, Surveillance, and Capacity teams).
3.2b: #, list of community OH events coordinated (country info captured in our questionnaire and now in EIDITH site and event form).

Please let me know if you have any questions and as always copy predict@ucdavis.edu.

Cheers,

David

From: "Andrew Clements" <aclements@usaid.gov>
Sent: 05/14/2019 2:02:12 AM (-07:00)
To: "Katherine Leasure" <kaleasure@ucdavis.edu>
Cc: "PREDICTMGT" <predictmgt@usaid.gov>; "Predict inbox" <predict@ucdavis.edu>; "Jonna Mazet" <Jkmazet@ucdavis.edu>
Subject: Re: PREDICT International Travel Requests

Approved.

Andrew Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
E-mail: aclements@usaid.gov

For more information on USAID's Emerging Pandemic Threats program, see: http://www.usaid.gov/ept2

On Tue, May 14, 2019 at 12:05 AM Katherine Leasure <kaleasure@ucdavis.edu> wrote:

Please find below international travel requests for your review and approval. Please let me know if you have any questions. Thanks!

1. O'Rourke, O'Rourke (USA): $579 airfare each/$196 (Davis) max daily per diem
2. Nga (USA): $1600 airfare/$196 (Davis) max daily per diem
3. [redacted] (USA): $1500 airfare/$196 (Davis), $174 (Tahoe City) max daily per diems

Travel Requests –

1. Metabiota would like to request travel approval for Tammie O'Rourke and Daniel O'Rourke to travel from Nanaimo, British Columbia, Canada to Davis, California USA from June 4-9, 2019 to meet with the UC Davis global team and assist with the training for the PREDICT-2 Data Conference being held.

Trip purpose: To meet with UCD global team and assist with training for the PREDICT-2 UCD Data Conference.

2. Wildlife Conservation Society (WCS) would like to request travel approval for Nguyen Thi Thanh Nga to travel from Ha Noi, Viet Nam to Davis, California, USA from June 4-15, 2019 to participate in the PREDICT-2 UCD Data Conference.

Trip purpose: The workshop will develop essential in-country capacity to understand, visualize, analyze and interpret country level PREDICT-2 data, and generate a framework for consistent and rigorous analysis for anticipated future publications. Nguyen Thi Thanh Nga will represent PREDICT Viet Nam on behalf of WCS.

3. UC Davis would like to request travel approval for [redacted] o travel from Dunblane, Scotland to Davis, California, USA from August 1-17, 2019 for meetings with Jonna Mazet and the UCD PREDICT global team, and to present at WDA 2019.

Trip purpose: [redacted] will travel to Davis to meet with Jonna Mazet and the PREDICT global team to finalize a country roll-out plan for the Spillover tool, and to prepare the accompanying documents, etc., that we will need to provide our international colleagues to see the Spillover tool used most efficiently. In addition, [redacted] will meet with the PREDICT global team to help prepare the final report and plan/contribute to sections on similar products and tools including the M&A section. [redacted] will also present the risk ranking application for PREDICT viruses at the Wildlife Disease Association Annual International Conference in Tahoe City, CA.

--
Katherine Leasure
HR/Payroll/Financial Assistant
One Health Institute
530-752-7526

--

From: Andrew Clements <aclements@usaid.gov>
To: Jonna Mazet <jkmazet@ucdavis.edu>
CC: David J Wolking <djwolking@ucdavis.edu>;PREDICTMGT <predictmgt@usaid.gov>
Sent: 9/19/2019 6:24:56 AM
Subject: Re: Quick request: language for report to Congress

Thanks. That'll do.

Andrew P. Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

On Sep 19, 2019, at 4:39 PM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:

Hi,
Thanks again for being there for the team in Bali. It meant a lot to all of us, especially me.

Okay, so none of this language looks right to me. Is it a GHSA report and thus just reporting on a subset of our work geographically and by dates? If so & you don't want to mess with any of the language except that which is bolded, I would change the bold stuff & what follows it to:
which are being characterized to determine which viruses are prioritized for further action according to the risks that they may pose to humans.

Hope that's helpful & that travel was easy back for all that came and supported & celebrated P-2 with us,
Jonna

On Thu, Sep 19, 2019 at 8:49 AM Andrew Clements <aclements@usaid.gov> wrote:
For a report to Congress, is the following bolded section okay? I didn't write it and it sounds kind of awkward

Thanks!

Surveillance: In 12 African and four Asian countries, USAID projects have tested samples from 15,118 animals and 2,316 humans for high priority viral families, such as corona-, filo-, flavi-, influenza-, and paramyxoviruses, which are associated with high consequence outbreaks, such as Ebola, Marburg, influenza, and Middle East Respiratory Syndrome (MERS). Results from Africa in 2018 include the identification of 17 new viruses and 26 existing viruses, **which will be prioritized to determine which viruses to characterize to** understand if they may pose risks to humans.

Andrew P. Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

From: "Dennis Carroll" REDACTED
Sent: 02/27/2020 11:53:05 AM (-08:00)
To: "Murray, Suzan" <MurrayS@si.edu>
Cc: "Aleksei Chmura" <chmura@ecohealthalliance.org>; "Alison Andre" <andre@ecohealthalliance.org>; "Cara Chrisman" <cchrisman@usaid.gov>; "Cheryl Bennett" <cheryl@gisaid.org>; "Eddy Rubin" <erubin@metabiota.com>; "Jennifer Gardy REDACTED REDACTED 'Jonna Mazet" <jkmazet@ucdavis.edu>; "Natalia Mercer" <nmercer@gvn.org>; "Oyewale Tomori" REDACTED "Peter Bogner" <peter@gisaid.org>; "Peter Daszak" <daszak@ecohealthalliance.org>; "Samtha Maher" <maher@ecohealthalliance.org>; "cbrechot@gvn.org" <cbrechot@gvn.org>; "graca@usf.edu" <graca@usf.edu>
Subject: Re: Invitation: GVP Board of Directors Monthly Call @ Monthly from 7pm to 8pm on the fourth Thursday (WAT) (REDACTED

As a follow up to our Board call, the person I was referring to as a possible panelist at the NYC event is Dr Syra Madad, head of NYC preparedness   Interestingly we were both just contacted by Entertainment Weekly to invite us to discuss Hollywood's take on pandemics -eg Contagion and World War Z. Boy the things I could say

World War Z notwithstanding, Dr Madad is terrific

On Thu, Feb 27, 2020 at 12:58 PM Murray, Suzan <MurrayS@si.edu> wrote:

> Dear All
>
> Congratulations on the Op Ed, Peter.
>
> For GVP today I am unfortunately not permitted to join just yet. Peter could you help us connect respective lawyers?
>
> Many thanks
>
> Suzan
>
> > On Feb 27, 2020, at 12:48 PM, Oyewale Tomori REDACTED wrote:
> >
> > External Email - Exercise Caution
> >
> > Dear Alison
> > Greetings is there another means of participating apart from the REDACTED number.....like webex zoom etc
> > i am unable to get tru to the number
> >
> > regards
> >
> > oyeweale
> > *Oyewale TOMORI*
> > Tel: REDACTED

On Thu, Feb 20, 2020 at 9:33 PM <maher@ecohealthalliance.org> wrote:

You have been invited to the following event.

GVP Board of Directors Monthly Call

When Monthly from 7pm to 8pm on the fourth Thursday West Africa Standard Time - Lagos

Calendar REDACTED

Who
- maher@ecohealthalliance.org - organizer
- Cara Chrisman
- Eddy Rubin
- Peter Daszak
- Cheryl Bennett
- REDACTED
- Aleksei Chmura
- Jonna Mazet
- cbrechot@gvn.org
- peter@gisaid.org
- Dennis Carroll
- Dr. Suzan Murray
- nmercer@gvn.org
- graca@usf.edu
- Alison Andre
- REDACTED

more details »

EcoHealth Alliance International Conference Line:

Number: +REDACTED

Passcode: REDACTED

Going REDACTED? All events in this series: **Yes** - **Maybe** - **No** more options »

Invitation from Google Calendar

You are receiving this email at the account REDACTED because you are subscribed for invitations on calendar REDACTED

To stop receiving these emails, please log in to https://www.google.com/calendar/ and change your notification settings for this calendar.

Forwarding this invitation could allow any recipient to send a response to the organizer and be added to the guest list, or invite others regardless of their own invitation status, or to modify your RSVP. Learn More.

--
Dr Dennis Carroll
President, Global Virome Project
Senior Fellow, Scowcroft Institute of International Affairs at the Bush School of Government and Public Service, Texas A&M University
Counselor and Advisor to the Faculty of Tropical Medicine at Mahidol University

mobile: REDACTED
email: REDACTED

From: alexandra zuber <alexandrazuber@atahealthstrategies.com>
To: Sam Halabi <sfh9@georgetown.edu>;Peter Daszak <daszak@ecohealthalliance.org>;Alison Andre <andre@ecohealthalliance.org>;William B. Karesh <karesh@ecohealthalliance.org>
CC: Jonna Mazet <jkmazet@ucdavis.edu>
Sent: 4/27/2020 12:26:03 PM
Subject: More board member interviews- SEAOHUN

Great news board development task team,
We had four more board members sign up for interviews in SEAOHUN region. Here is the snapshot below. Sam, we all owe you comments ASAP on the revised questionnaires (thank you for sending)...

Peter you signed up for April 29, so you now have an interview with **Abdul Rahman Omar**

- **I will schedule yours and include Billy and Sam for interest**

Sam you are signed up for April 30, and May 1, so you have: **Michael Tee** and **Suwat** (not featured in the Doodle) and **Bui Tran Anh Dao**

- **Sam, can you schedule yours since you are already well versed in this and in saving Zoom videorecordings? Remember to include Tracey.**

I am signed up for May 4, so I will see if Le Thi Huong can reschedule until this date. Or **Billy,** is this one you'd like to lead?

Let me know if you have any questions.

Thanks all,
Alexandra

Table Calendar

	Apr 27 MON 7:00 AM 8:00 AM	Apr 27 MON 8:00 AM 9:00 AM	Apr 28 TUE 7:00 AM 8:00 AM	Apr 28 TUE 8:00 AM 9:00 AM	Apr 29 WED 7:00 AM 8:00 AM	Apr 29 WED 8:00 AM 9:00 AM	Apr 30 THU 7:00 AM 8:00 AM	Apr 30 THU 8:00 AM 9:00 AM	May 1 FRI 7:00 AM 8:00 AM	May 1 FRI 8:00 AM 9:00 AM	May 4 MON 7:00 AM 8:00 AM	May 4 MON 8:00 AM 9:00 AM	May 5 TUE 7:00 AM 8:00 AM	May 5 TUE 8:00 AM 9:00 AM
5 participants	✓2	✓2	✓0	✓1	✓0	✓2	✓0	✓2	✓1	✓1	✓1	✓1	✓0	✓0
Alexandra Zuber	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Abdul Rahman Omar						✓		✓						
Sathirakorn Pongpa...	✓										✓			
Bui Tran Anh Dao									✓	✓				
Le Thi Huong	✓	✓												
Michael Tee		✓		✓		✓		✓				✓	?	?

Alexandra Zuber, MPP, DrPH
Founder and CEO, Ata Health Strategies, LLC
Email: alexandrazuber@atahealthstrategies.com
Phone: +1 (617) 680-3950
LinkedIn: alexandrazuber/
Website: www.atahealthstrategies.com
Twitter: @alexandrazuber

From: alexandra zuber <alexandrazuber@atahealthstrategies.com>
To: REDACTED "wasmith@ucdavis.edu" <wasmith@ucdavis.edu>, REDACTED "mcrane@usaid.gov" <mcrane@usaid.gov>, "margaritamartins@berkeley.edu" <margaritamartins@berkeley.edu>, "f.castillo@berkeley.edu" <f.castillo@berkeley.edu>, "oromero@haas.berkeley.edu" <oromero@haas.berkeley.edu>, Elizabeth Leasure <ealeasure@ucdavis.edu>, "apereira@usaid.gov" <apereira@usaid.gov>, Kevin Olival <olival@ecohealthalliance.org>, REDACTED Matthew Blake <mblake@ucdavis.edu>, Peter Daszak <daszak@ecohealthalliance.org>, "tgoldstein@ucdavis.edu" <tgoldstein@ucdavis.edu>, "clouisduthil@usaid.gov" <clouisduthil@usaid.gov>, "jkmazet@ucdavis.edu" <jkmazet@ucdavis.edu>, "sfh9@georgetown.edu" <sfh9@georgetown.edu>, "karesh@ecohealthalliance.org" <karesh@ecohealthalliance.org>, Nichapha Chinawong REDACTED Juthamanee Areeya REDACTED REDACTED REDACTED
Subject: Pre-reads: SEAOHUN Virtual Workshop: OCA @ Thu May 14, 2020 9pm - 11pm (EDT) (alexandrazuber@atahealthstrategies.com)
Sent: Thu, 14 May 2020 14:59:54 +0000
2020-05-15 Self-rated OCA of SEAOHUN Secretariat.pdf
2020-05-15 Self-rated OCA of SEAOHUN Secretariat.pptx

Hello all,
Please find two pre-reads for our meeting tonight with SEAOHUN on the OCA:

1. SEAOHUN's self assessment on the OCA tool
2. Their PPT presentation they will deliver tonight to guide our discussion.

We look very forward!

Sincerely,
Alexandra, Omar, Federico, and Margarita

Alexandra Zuber, MPP, DrPH
Founder and CEO, Ata Health Strategies, LLC
Email: alexandrazuber@atahealthstrategies.com
Phone: +1 (617) 680-3950
LinkedIn: alexandrazuber/
Website: www.atahealthstrategies.com
Twitter: @alexandrazuber

From: REDACTED
Sent: Wednesday, May 6, 2020 10:37 AM
To: REDACTED wasmith@ucdavis.edu <wasmith@ucdavis.edu>; REDACTED; mcrane@usaid.gov <mcrane@usaid.gov>; margaritamartins@berkeley.edu <margaritamartins@berkeley.edu>; f.castillo@berkeley.edu <f.castillo@berkeley.edu>; oromero@haas.berkeley.edu <oromero@haas.berkeley.edu>; Elizabeth Leasure <ealeasure@ucdavis.edu>; apereira@usaid.gov <apereira@usaid.gov>; Kevin Olival <olival@ecohealthalliance.org>; REDACTED alexandra zuber <alexandrazuber@atahealthstrategies.com>; Matthew Blake <mblake@ucdavis.edu>; Peter Daszak <daszak@ecohealthalliance.org>; tgoldstein@ucdavis.edu <tgoldstein@ucdavis.edu>; clouisduthil@usaid.gov <clouisduthil@usaid.gov>; jkmazet@ucdavis.edu <jkmazet@ucdavis.edu>; sfh9@georgetown.edu <sfh9@georgetown.edu>; karesh@ecohealthalliance.org <karesh@ecohealthalliance.org>
Subject: Invitation: SEAOHUN Virtual Workshop: OCA @ Thu May 14, 2020 9pm - 11pm (EDT) (alexandrazuber@atahealthstrategies.com)
When: Thursday, May 14, 2020 9:00 PM-11:00 PM.
Where: REDACTED

You have been invited to the following event.

SEAOHUN Virtual Workshop: OCA

When Thu May 14, 2020 9pm – 11pm Eastern Time - New York

Where [redacted] (map)

Calendar alexandrazuber@atahealthstrategies.com

Who
- [redacted] - organizer
- wasmith@ucdavis.edu
- [redacted]
- mcrane@usaid.gov
- margaritamartins@berkeley.edu
- f.castillo@berkeley.edu
- oromero@haas.berkeley.edu
- Elizabeth Leasure
- apereira@usaid.gov
- Kevin Olival
- [redacted]
- alexandrazuber@atahealthstrategies.com
- Matthew Blake
- Peter Daszak
- tgoldstein@ucdavis.edu
- clouisduthil@usaid.gov
- jkmazet@ucdavis.edu
- sfh9@georgetown.edu
- karesh@ecohealthalliance.org

more details »

Dear all,
We are delighted to invite you to the OHW-NG Objective 3 virtual workshop regarding the USAID Organizational Capacity Assessment (OCA) Tool. This is the first in a series of workshops we will be holding with the SEAOHUN Secretariat in May.

This tool is designed to help organizations benchmark their organizational capacity across multiple domains, and identify priorities for improving organizational performance. While traditionally used for organizations that already receive direct USAID funding, we believe conducting a baseline and understanding the needs and priorities now in Year 1 will set the scene for improved capacity-building over the full five years of the OHW-NG initiative.

SEAOHUN will send an agenda and slides in advance of our meeting to review. The objectives of our workshop together are:

- Provide a forum for SEAOHUN to share its self assessment of strengths and weaknesses on key organizational capability domains and establish a baseline of organizational capacity
- Facilitate dialogue between global team and SEAOHUN on areas of improvement in organizational capacity
- Identify priority areas for capacity-building in the short and medium terms under OHW-NG

We are looking very forward to it.

Sincerely,
Alexandra, Omar, Federico, and Margarita
Alexandra Zuber is inviting you to a scheduled Zoom meeting.

Join Zoom Meeting

REDACTED

Meeting ID: REDACTED
Password: REDACTED
One tap mobile
REDACTED US (Germantown)
US (Chicago)

Dial by your location
REDACTED (Germantown)
(Chicago)
(New York)
(Tacoma)
(Houston)
(San Jose)
Meeting ID REDACTED
Password:
Find your local number: REDACTED
Going (alexandrazuber@atahealthstrategies.com)? **Yes** - **Maybe** - **No** more options »

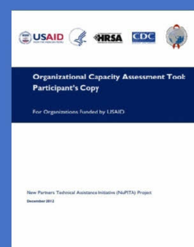
SEAOHUN
Southeast Asia One Health University Network

Organizational Capacity Assessment Tool

15 May 2020

This presentation is made possible by the generous support of the American people through the United States Agency for International Development (USAID).
The contents are the responsibility of One Health Workforce implementing partners and do not necessarily reflect the views of USAID or the United States Government.

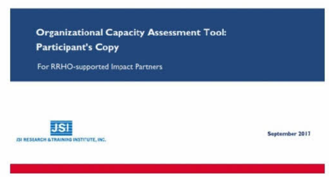
Assessment



Organizational Capacity Assessment Tool:
Participant's Copy

For Organizations Funded by USAID

New Partners Technical Assistance Initiative (NuPITA) Project
December 2012



Organizational Capacity Assessment Tool:
Participant's Copy

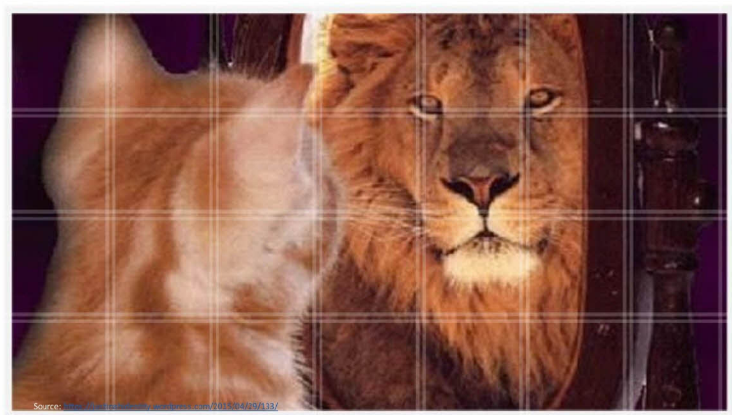
For RRHO-supported Impact Partners

JSI RESEARCH & TRAINING INSTITUTE, INC.

September 2017

Small & Overstretched → Multiple roles
Limited Views → May change with growing team

Documentation vs Implementation


Source: wordpress.com/2015/04/29/133/

EXAMPLE

Administration

Objective: To assess the organization's capacity to develop and apply policies and procedures, the existence and quality of its administrative systems and its staff knowledge of the systems.

Operational Policies, Procedures, and Systems

Subsection Objective: To assess the availability of and adherence to operational policies.

Resources: policy and procedures manual, anonymous staff questionnaires, related payment vouchers

Operational Policies, Procedures, and Systems ●			
1	**2**	**3**	**4**
The organization has • No documented operational policies and procedures	The organization has • Documented some operational policies and procedures, but they are incomplete or not compliant with national and donor regulations • Policies and procedures that are not consistently adhered to • Not oriented or trained staff in the policies and procedures	The organization has • Documented most or all operational policies and procedures and they are compliant with national and donor regulations • Policies and procedures that are known but not consistently adhered to • Oriented or trained staff in the policies and procedures • No process for regularly reviewing and updating operational policies and procedures	The organization has • Complete and appropriate operational policies and procedures • Policies and procedures that are known and understood by staff • Policies and procedures that are consistently adhered to, reviewed and updated

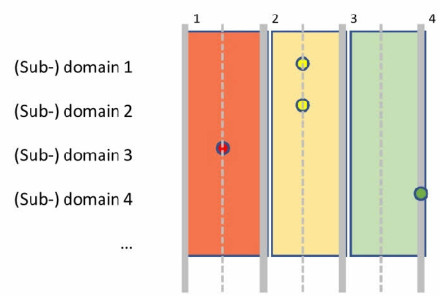
3 manuals are available and effective in 2020, including Staff Manual, Financial Management Manual, and Procurement Manual.

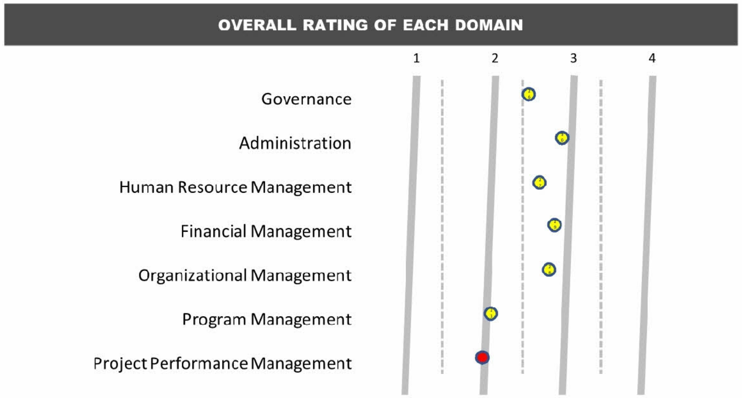
7 OCA Domains

TUCK
2. Administration
3. Human Resource Management
4. Financial Management

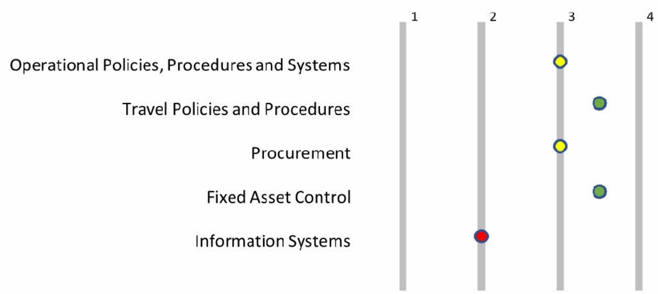
PAT
1. Governance
5. Organizational management
6. Program Management
7. Project performance management

RATING of EACH DOMAIN & SUB-DOMAIN




OVERALL RATING OF EACH DOMAIN
1
2
3
4
Governance
Administration
Human Resource Management
Financial Management
Organizational Management
Program Management
Project Performance Management

2. ADMINISTRATION



ADMIN-
ISTRATION

Technical Assistance

- Guide and support to complete all necessary manuals/policies/procedures (for all domains) required to be a direct recipient of USAID funds, including information system policies and procedures, procurement plan
- Review the fixed-asset register and suggest ways to address gaps if existed
- Train SEAOHUN/OHUNs on procurement regulations

Continued Improvement by the Secretariat

- Review and update manuals/policies/procedures at least once a year
- (Re)orient staff members on manuals/policies/procedures

3. HUMAN RESOURCE MANAGEMENT

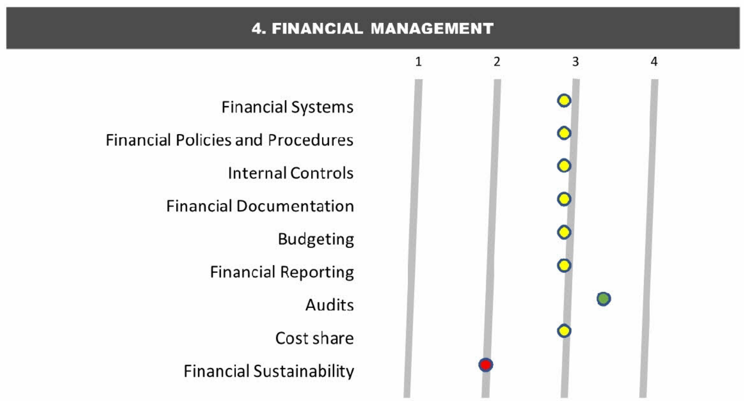
HUMAN
RESOURCE
MGMT

Technical Assistance

- Recommend an appropriate organizational structure both short- and long-term given limited resources.
- Guide and support to improve HR policies and guidelines including retention of staff
- Support setting aside fund for professional development and team building

Continued Improvement by the Secretariat

- Develop relevant forms to support HR process
- Review/revise JDs of all staff members


4. FINANCIAL MANAGEMENT
1
2
3
4
Financial Systems
Financial Policies and Procedures
Internal Controls
Financial Documentation
Budgeting
Financial Reporting
Audits
Cost share
Financial Sustainability

FINANCIAL MGMT

Technical Assistance

- Review/recommend/train on financial system/software for SEAOHUN/OHUNs
- Train SEAOHUN/OHUNs on internal controls, financial procedures, budgeting, and reporting for common understanding
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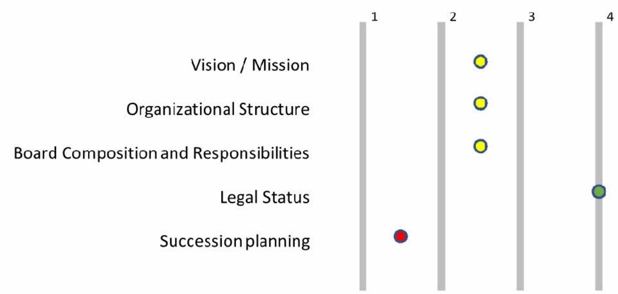
1. GOVERNANCE



GOVERNANCE

Technical Assistance

- Motivate/transform Executive Board to help drive SEAOHUN policies and resource mobilization effort (value of SEAOHUN/Secretariat, clear TOR, # of members, voting vs non-voting, terms and rotation, bylaws, having an advisory board, co-contribution, required changes at OHUN Board, etc.)
- Develop the succession plan (OHUNs may need one, too)
- Guide on whether to establish new NCOs

Continued Improvement by the Secretariat

- (Re)orient Board members of their roles and responsibilities


5. ORGANIZATIONAL MANAGEMENT
1
2
3
4
Strategic Planning
Resource Mobilization
Operational Plan
Communication Strategy
Change Management
Knowledge Management
Stakeholder Involvement
Internal Communication
Decision Making

ORGANIZATIONAL MANAGEMENT

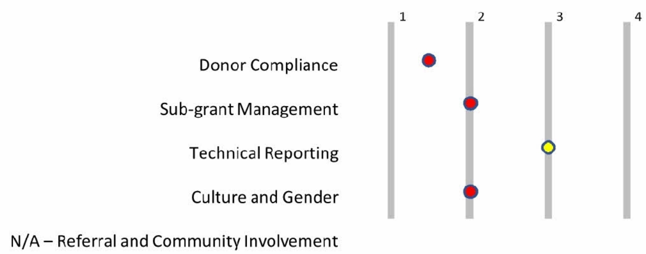
Technical Assistance

- Diversify funding portfolio through engagement of Board, Secretariat, OHUNs (teamwork) → shifting from project focus to organizational focus
- Foster teamwork among Secretariat and NCOs (better understanding one another; past – no budget to strengthen collaboration)
- Review and recommend actions to improve organizational management

Continued Improvement by the Secretariat

- Foster teamwork, improve processes, and delegation of tasks within the doubling size of the Secretariat

6. PROGRAM MANAGEMENT



PROGRAM
MGMT

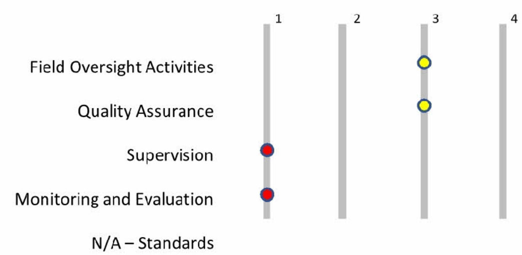
Technical Assistance

- Mentor/guide/train the Secretariat on donor compliance, sub-grant management, culture and gender

Continued Improvement by the Secretariat

- Enhance Secretariat capacity and skills through training and learning-by-doing (young team)

7. PROJECT PERFORMANCE MANAGEMENT



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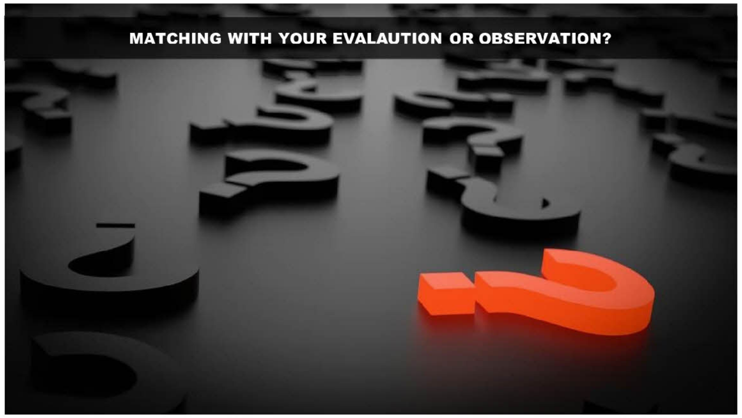
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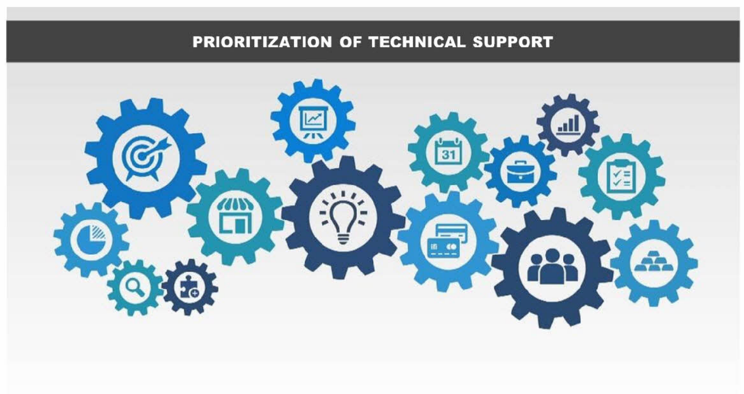
PRIORITIZATION OF TECHNICAL SUPPORT

Short-term
(Y1-Y3)

Every aspects necessary to pass NUPAS

1. **Documentation** – Guide and support to complete all necessary manuals/policies/procedures required to be a direct recipient of USAID funds
2. **Staffing** – Recommend an appropriate organizational structure, budget and strategies to address retention
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---

- Review the fixed-asset register and suggest ways to address gaps if existed

ONGOING

Required ongoing efforts

- Transform the Board and ways to engage members (centralized vs de-centralized) to increase the likelihood of sustainability
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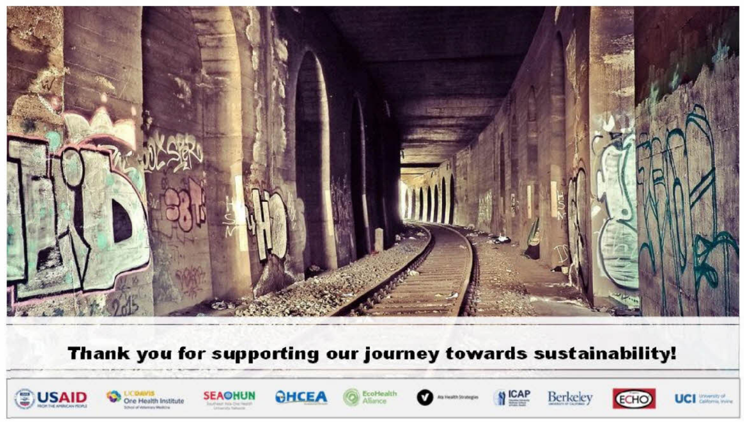
- Continuous improvement on reviewing, mentoring, and guiding the Secretariat for success to address our gaps.

Long-term
(Y4-Y5)

Can be addressed in later years

- Diversify funding (byproduct of a good resource mobilization team if Board can be transformed)


Thank you for supporting our journey towards sustainability!
USAID
One Health Institute
SEAOHUN
OHCEA
EcoHealth Alliance
ICAP
Berkeley
ECHO
UCI

SEAOHUN
Southeast Asia One Health University Network

Organizational Capacity Assessment Tool

15 May 2020

This presentation is made possible by the generous support of the American people through the United States Agency for International Development (USAID).
The contents are the responsibility of One Health Workforce implementing partners and do not necessarily reflect the views of USAID or the United States Government.

Assessment

Organizational Capacity Assessment Tool: Participant's Copy

For Organizations Funded by USAID

New Partners Technical Assistance Initiative (NuPITA) Project

December 2012

Organizational Capacity Assessment Tool: Participant's Copy

For RRHO-supported Impact Partners

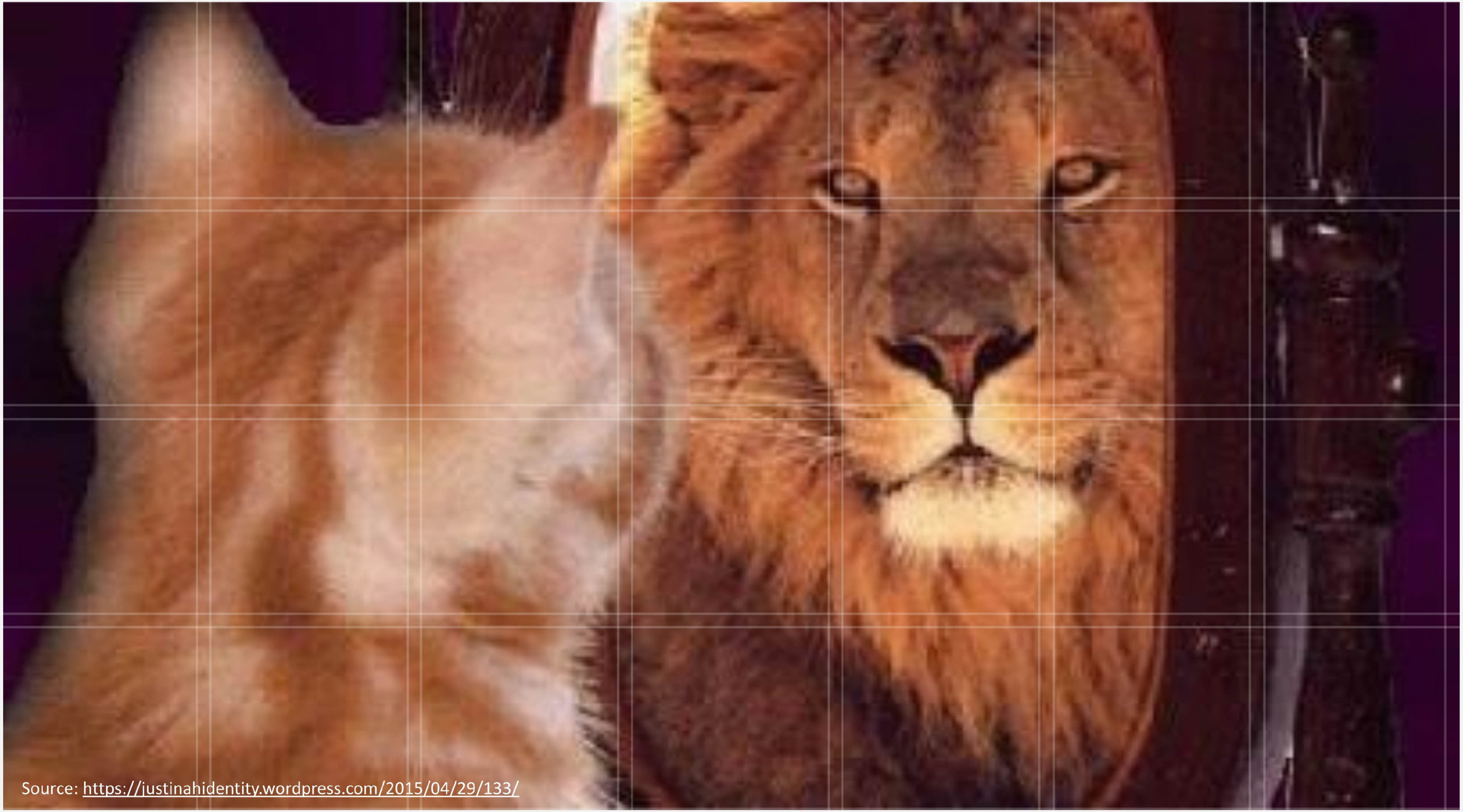
JSI RESEARCH & TRAINING INSTITUTE, INC.

September 2017

Small & Overstretched → Multiple roles

Limited Views → May change with growing team

Documentation vs Implementation



Source: https://justinahidentity.wordpress.com/2015/04/29/133/

UCDUSR0013352

EXAMPLE

Administration

Objective: To assess the organization's capacity to develop and apply policies and procedures, the existence and quality of its administrative systems and its staff knowledge of the systems.

Operational Policies, Procedures, and Systems

Subsection Objective: To assess the availability of and adherence to operational policies.

Resources: policy and procedures manual, anonymous staff questionnaires, related payment vouchers

Operational Policies, Procedures, and Systems ●			
1	2	3	4
The organization has • No documented operational policies and procedures	The organization has • Documented some operational policies and procedures, but they are incomplete or not compliant with national and donor regulations • Policies and procedures that are not consistently adhered to • Not oriented or trained staff in the policies and procedures	The organization has • Documented most or all operational policies and procedures and they are compliant with national and donor regulations • Policies and procedures that are known but not consistently adhered to • Oriented or trained staff in the policies and procedures • No process for regularly reviewing and updating operational policies and procedures	The organization has • Complete and appropriate operational policies and procedures • Policies and procedures that are known and understood by staff • Policies and procedures that are consistently adhered to, reviewed and updated

3 manuals are available and effective in 2020, including Staff Manual, Financial Management Manual, and Procurement Manual.

7 OCA Domains

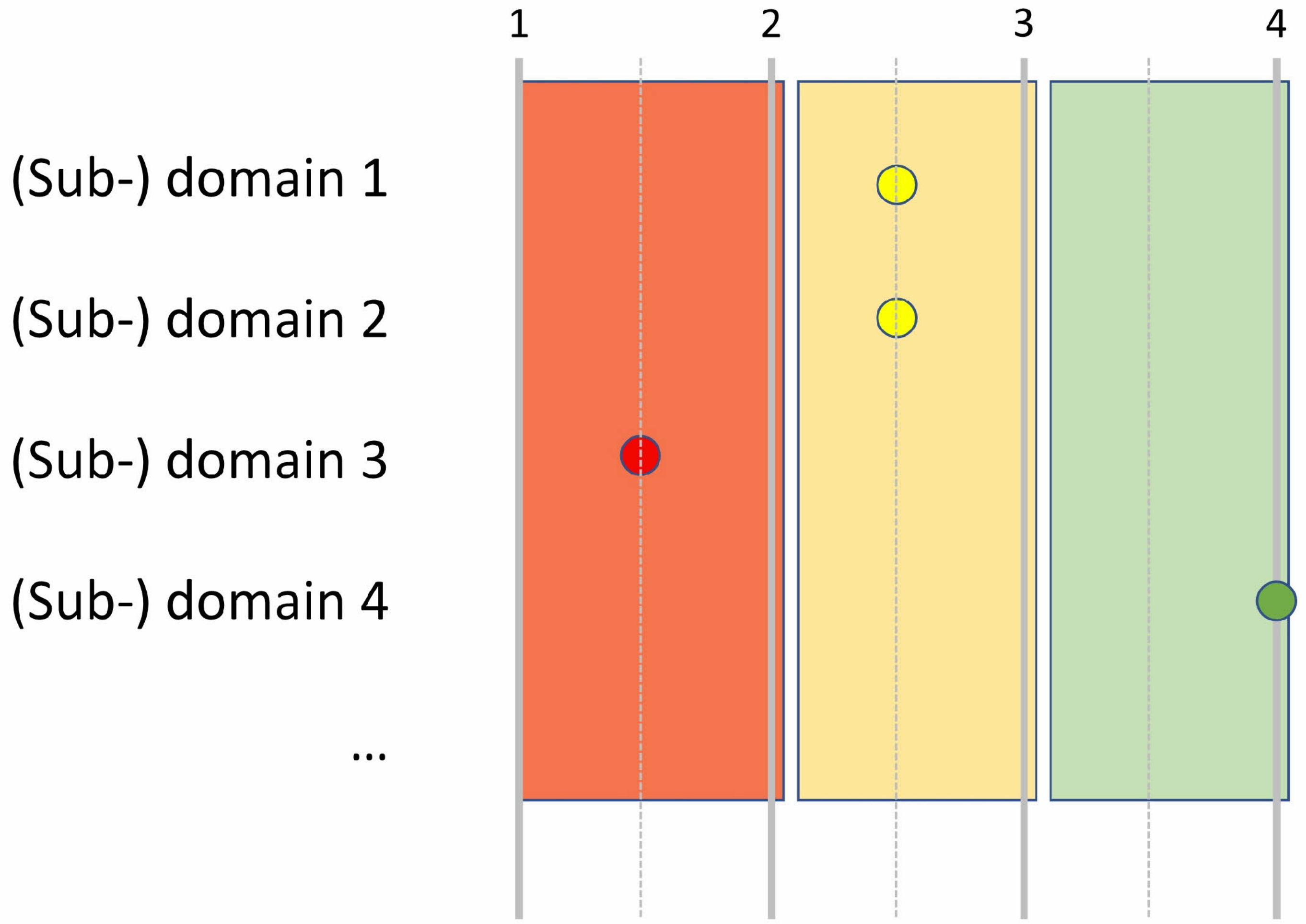
TUCK

2. Administration
3. Human Resource Management
4. Financial Management

PAT

1. Governance
5. Organizational management
6. Program Management
7. Project performance management

RATING of EACH DOMAIN & SUB-DOMAIN



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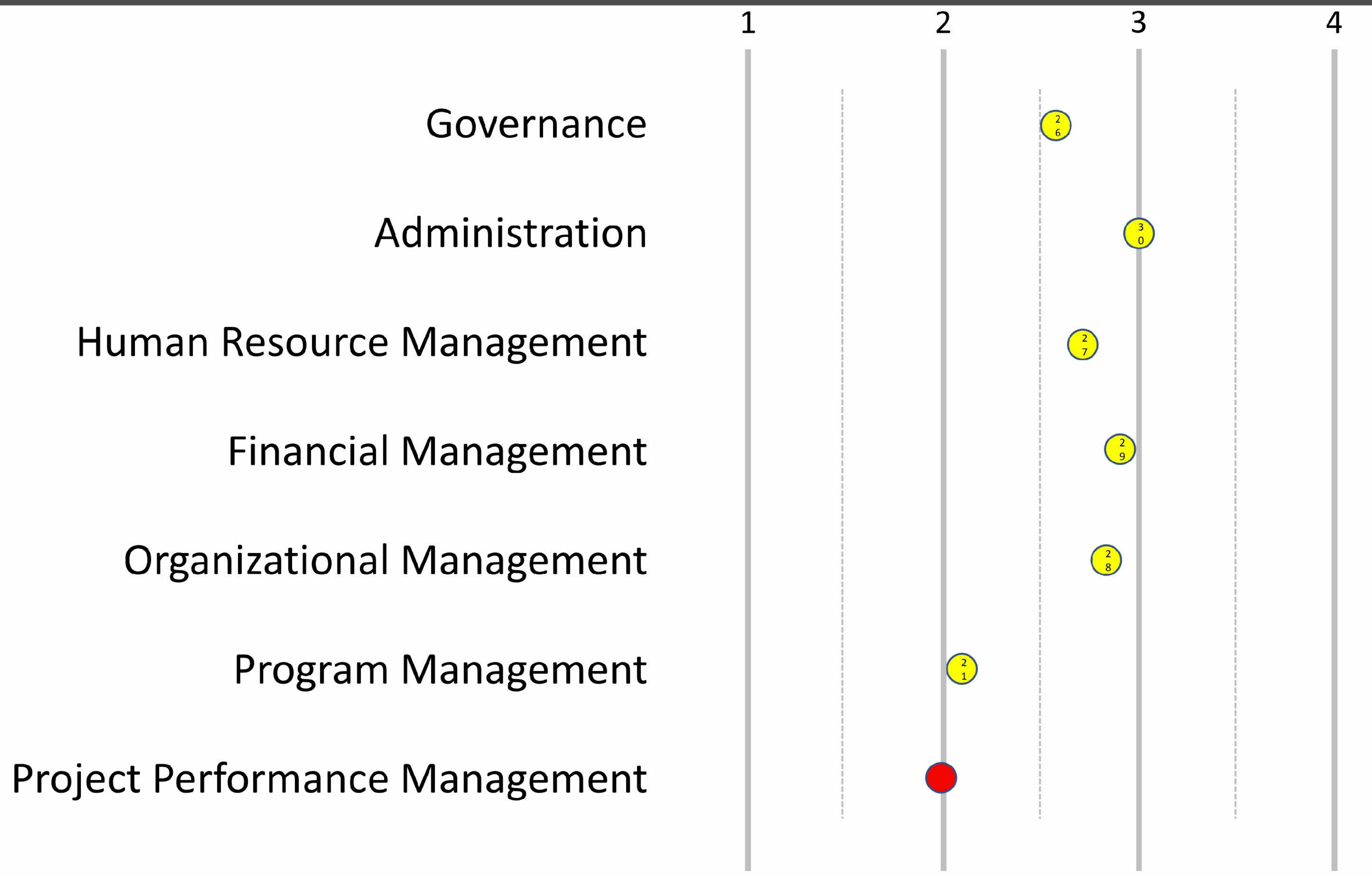

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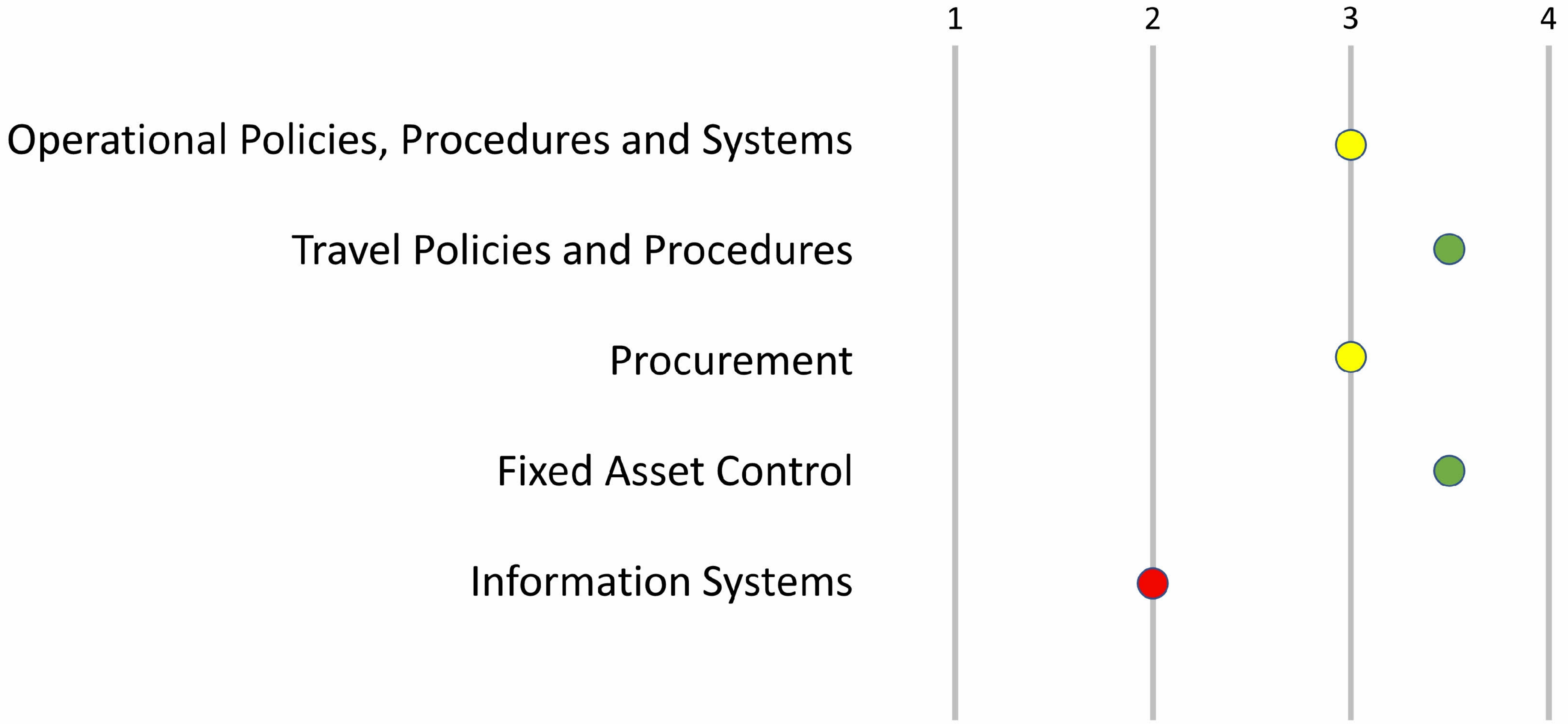

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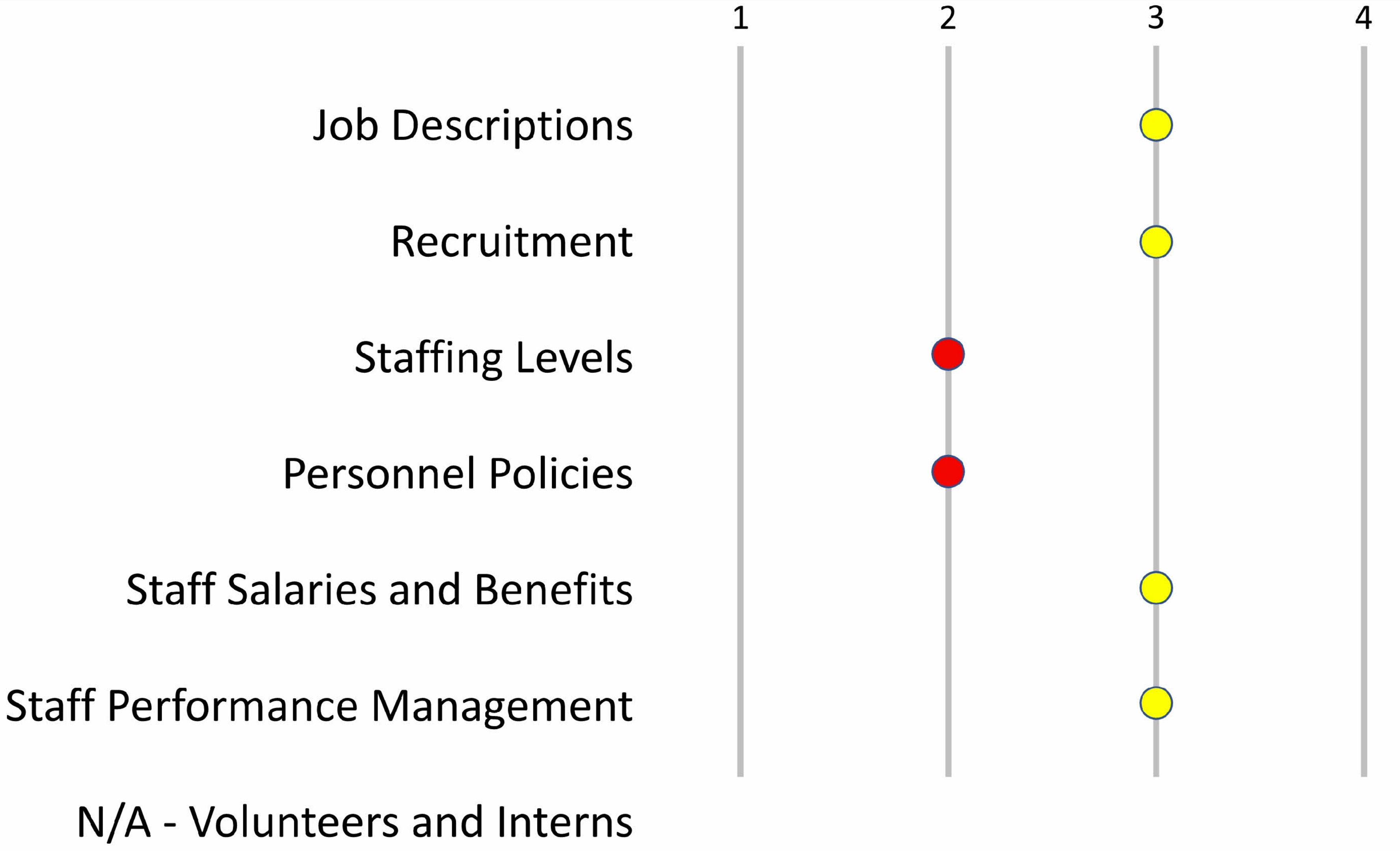

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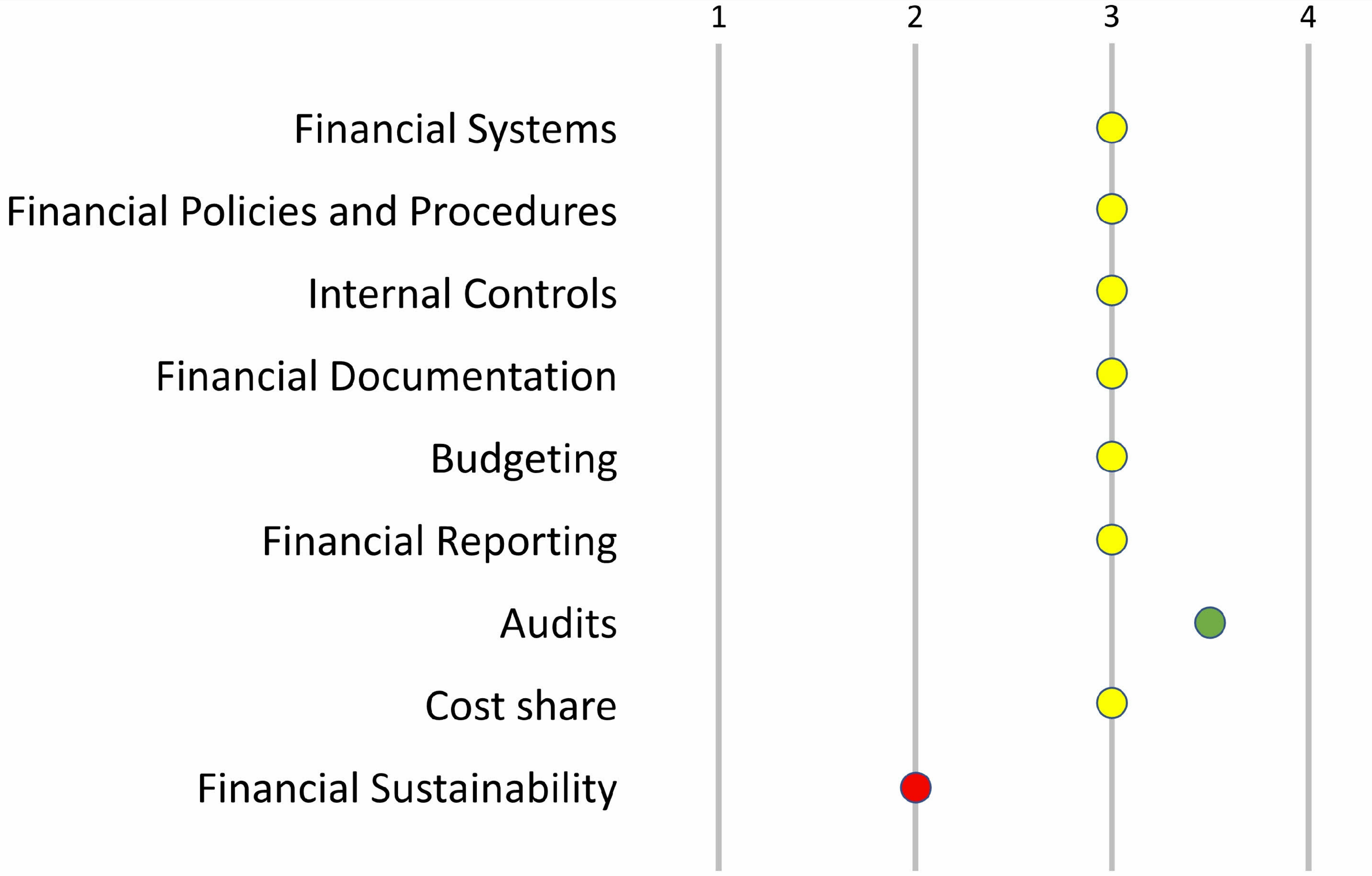

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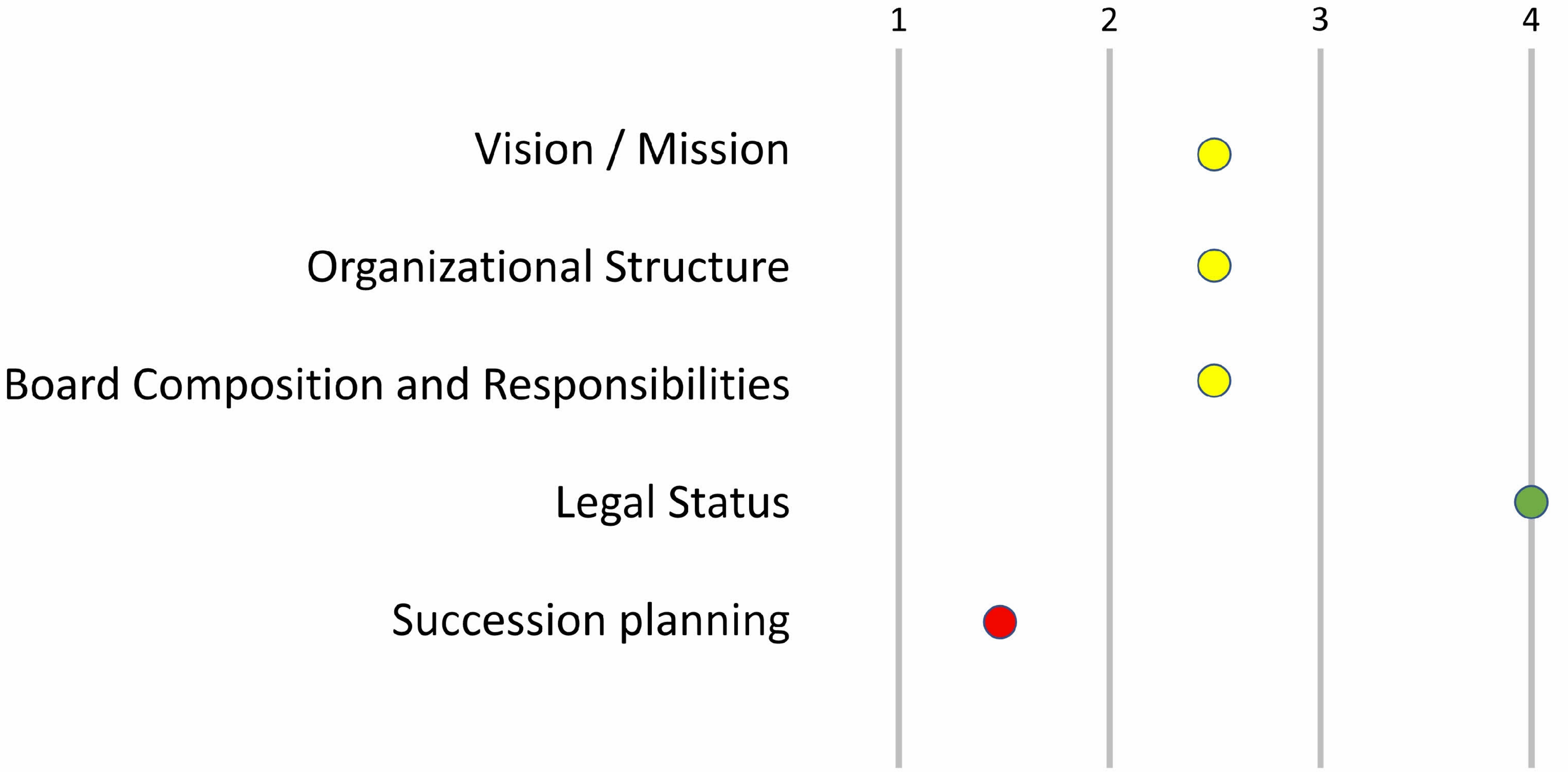

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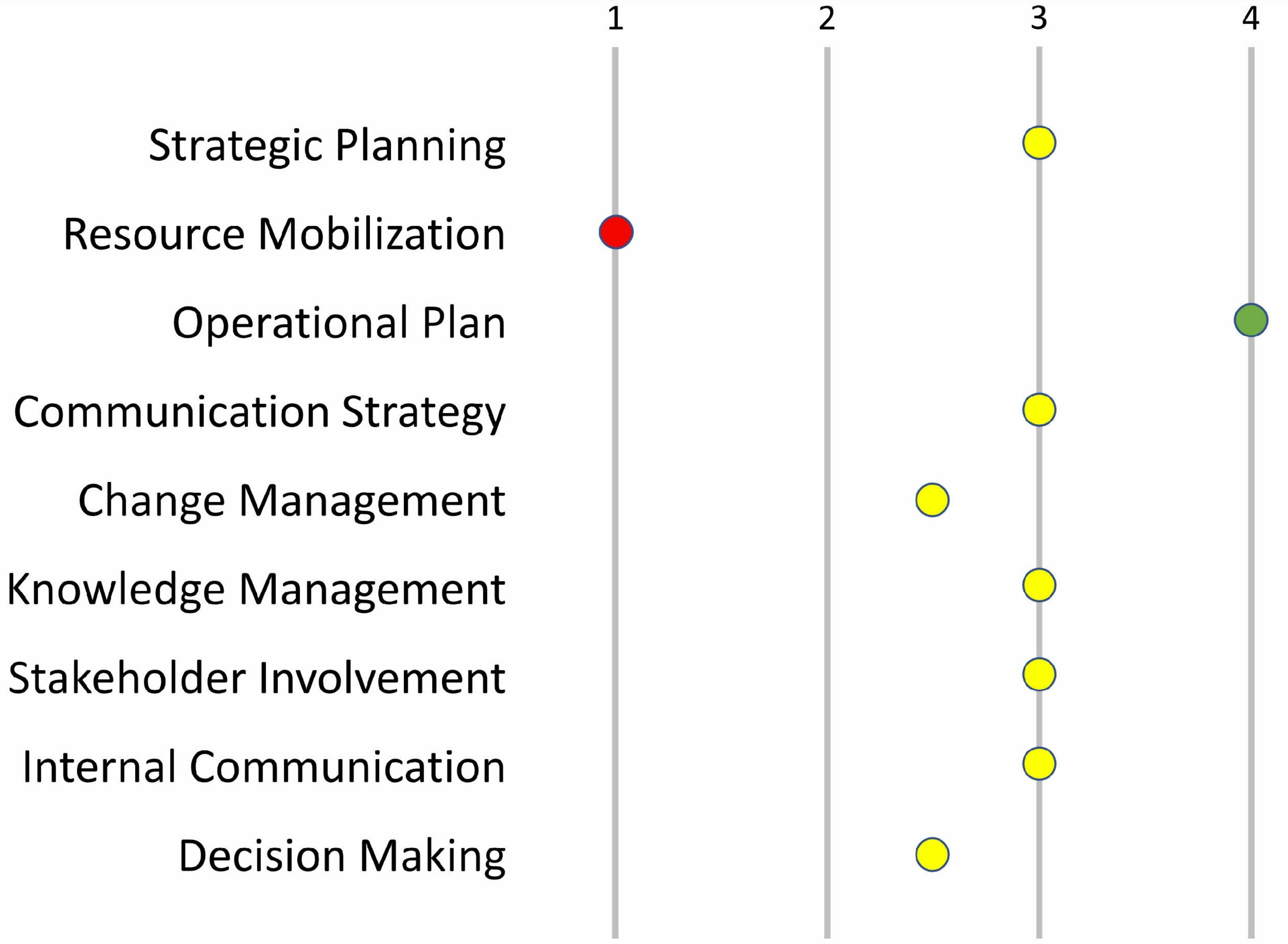

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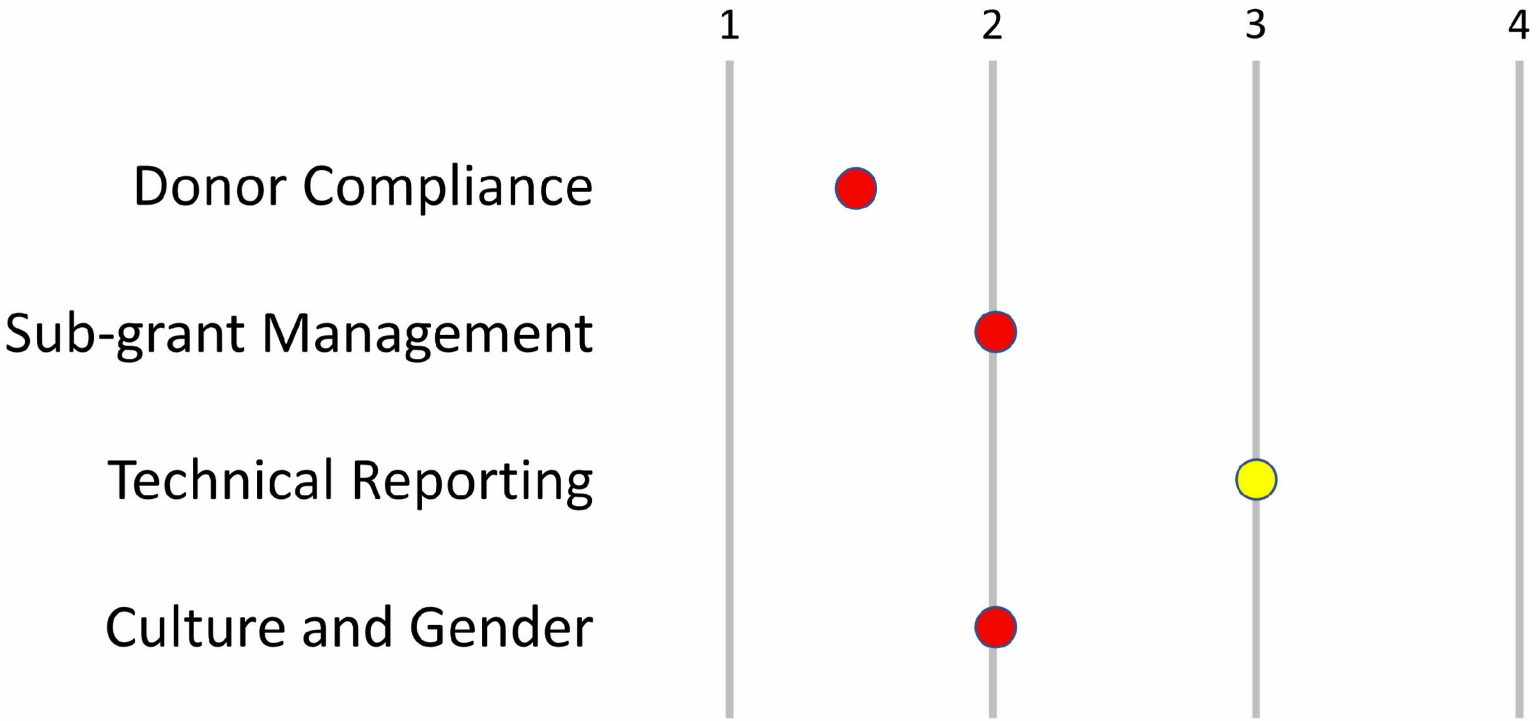

N/A – Referral and Community Involvement

PROGRAM MGMT

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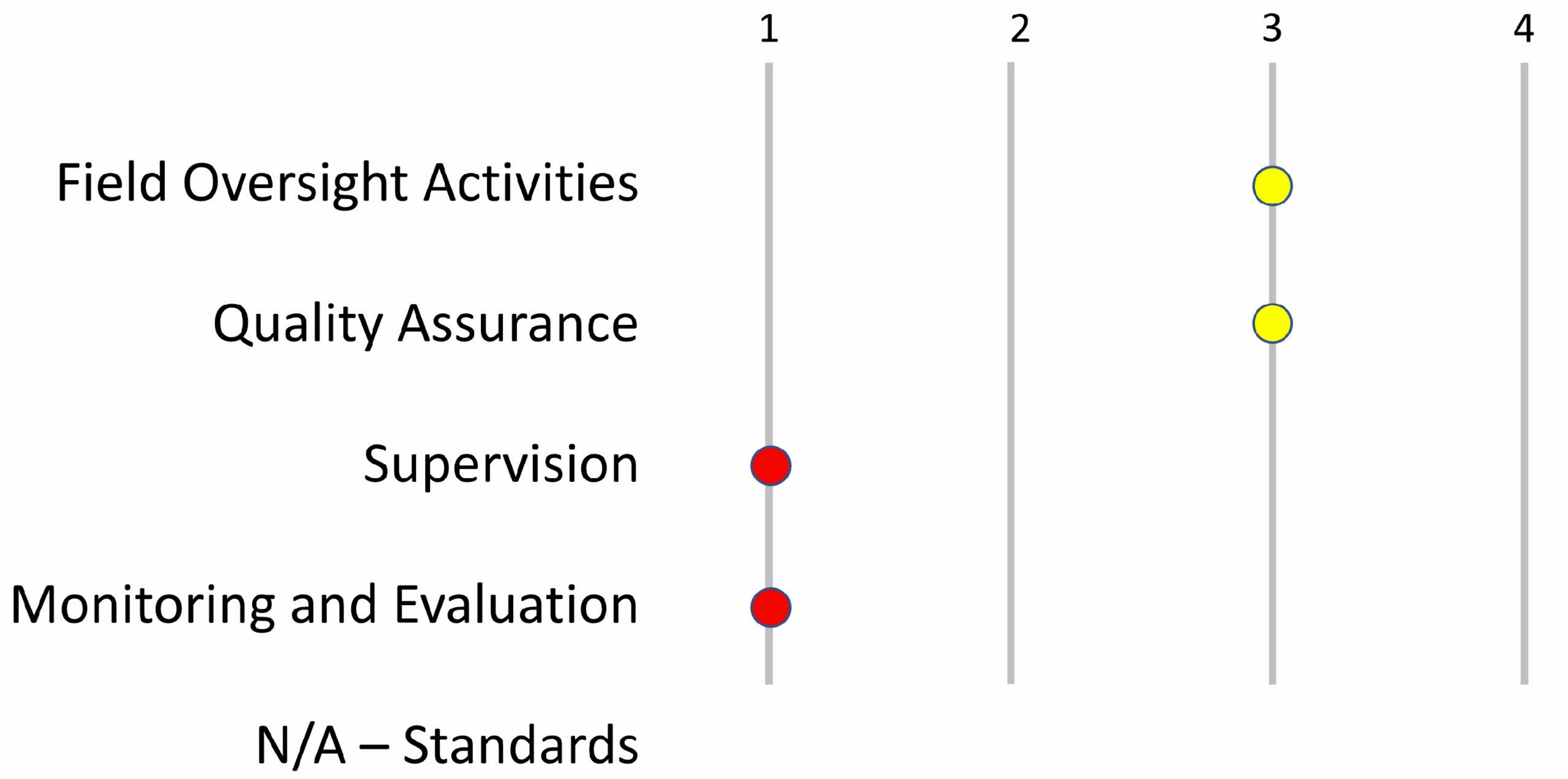

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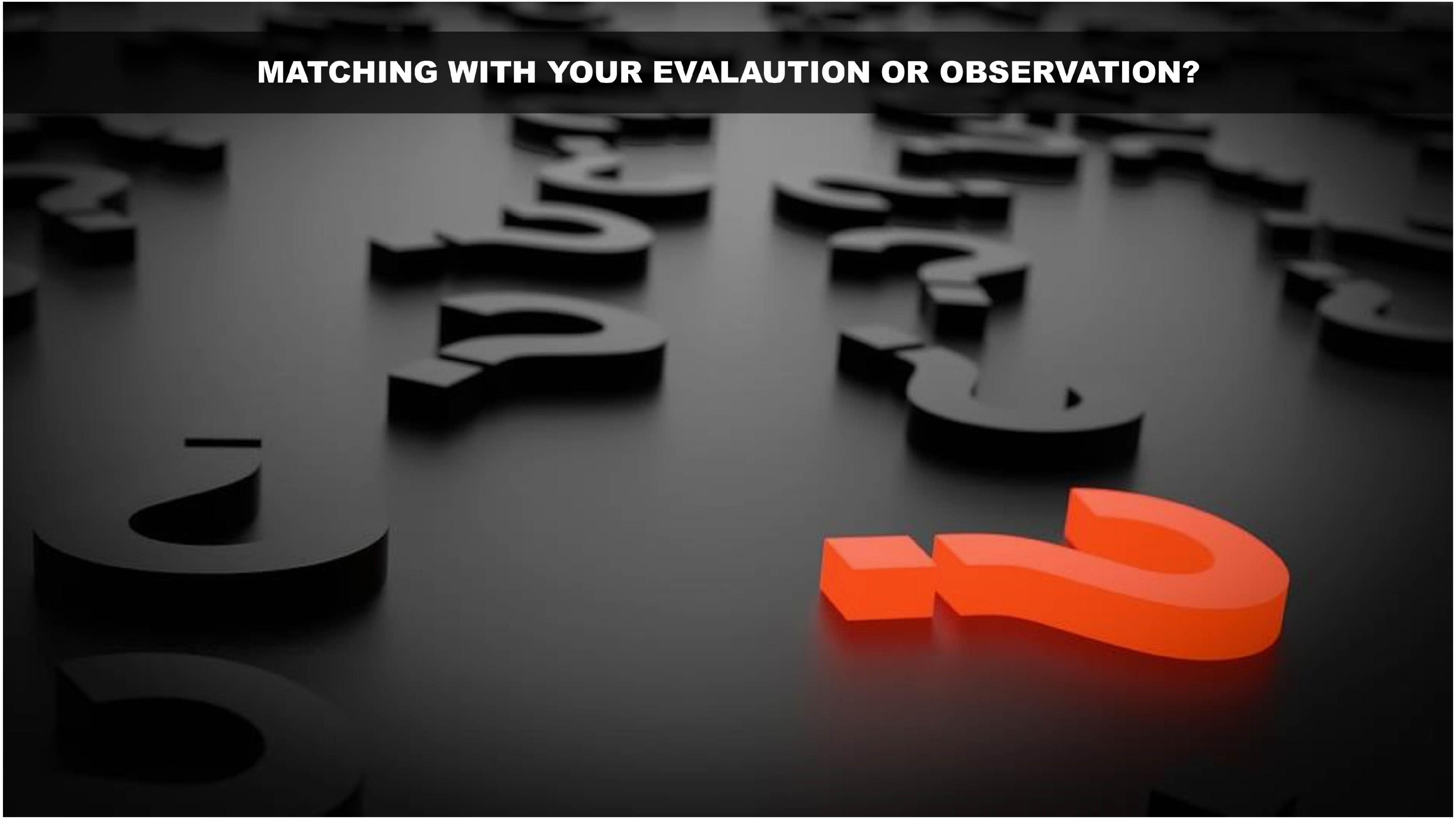
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Thank you for supporting our journey towards sustainability!

USAID FROM THE AMERICAN PEOPLE | UCDAVIS One Health Institute School of Veterinary Medicine | SEAOHUN Southeast Asia One Health University Network | OHCEA | EcoHealth Alliance | Ata Health Strategies | ICAP Columbia University Mailman School of Public Health | Berkeley University of California | Project ECHO | UCI University of California, Irvine

From: "Jonna Mazet" <jkmazet@ucdavis.edu>
Sent: 06/09/2020 5:40:28 PM (-07:00)
To: "Dennis Carroll" <[redacted]>
Subject: Re: project idea and introduction
Attachments: KAA_Initiative_MOU_DRAFT_June9_2020.docx

FYI -- request for MOU consideration with the KAA rainforest group (XPrize) that I mentioned. No $$$ in this one, just collaboration. How would you like to proceed?
J

On Tue, Jun 9, 2020 at 2:10 PM Juan Carlos Castilla Rubio <[redacted]> wrote:

Dear Jonna,

Many thanks indeed for agreeing to become a founding partner in the KAA Initiative !

As per our discussions over the last few weeks / months our overall collaborative objective is to develop, test and operate a family of autonomous aerial, terrestrial, aquatic systems equipped with multiple advanced sensor payloads to “autonomously sense, predict, and discover life in the Amazon, understand their genomics, the biochemical processes involved, the biological functions at play, their complex interrelationships and the risks of pathogen spillover events leading to future pandemics in the context of the Amazon’s ecosystems disruptions underway” - for future deployment of autonomous sensing robots for biological discovery in the Amazon at scale, so that an inclusive bioeconomy in the Amazon can be unleashed for the benefit their peoples whilst preserving life in the Amazon for future generations.

Also, as per our past discussions and to formalize your Center/ institution participation in the KAA Initiative would very much appreciate your review and comments (using track changes) back to the draft KAA Initiative MOU attached by end of next week at the latest. It is important to note that nothing in this MOU will be construed as creating binding legal obligations between the parties to the KAA Initiative but rather as a broad statement of intent which sets forth the general basis upon which the parties wish to proceed in their collaboration.

Many thanks indeed and hope all is well with you and your colleagues.

Look forward to hearing from you.

Best, juan carlos

From: Jonna Mazet <jkmazet@ucdavis.edu>
Date: Friday, May 29, 2020 at 3:26 PM
To: Juan Carlos Castilla Rubio [redacted]
Cc: Cristina Davis <cedavis@ucdavis.edu>, Christine Kreuder Johnson <ckjohnson@ucdavis.edu>, Mary Radford <maradford@ucdavis.edu>, Marcy Uhart <muhart@ucdavis.edu>
Subject: Re: project idea and introduction

Great news -- our leadership team gave the affiliation a thumbs-up! I'm just working to get you the comprehensive paragraph, as requested. Will have that to you today.

Onward & upward!
Jonna

Jonna AK Mazet, DVM, MPVM, PhD

Professor of Epidemiology & Disease Ecology

Executive Director, One Health Institute

Director, One Health Workforce – Next Generation of USAID Emerging Threats Division

Director Emeritus, PREDICT Project of USAID Emerging Threats Division

Board of Directors, Global Virome Project

School of Veterinary Medicine

University of California, Davis

Institute for Global Health Sciences

University of California, San Francisco

For scheduling and logistical issues, please contact:

Ms. Mary Radford

maradford@ucdavis.edu

On Fri, May 29, 2020 at 6:57 AM Juan Carlos Castilla Rubio [redacted] wrote:

Dear Jonna

Really enjoyed our conversation earlier this week with you and your colleagues– look forward to your input ref joining our KAA consortia later today ?

Best, juan carlos

From: Jonna Mazet <jkmazet@ucdavis.edu>
Date: Friday, May 22, 2020 at 6:50 PM
To: Cristina Davis <cedavis@ucdavis.edu>
Cc: Christine Kreuder Johnson <ckjohnson@ucdavis.edu>, Juan Carlos Castilla Rubio <REDACTED>, Mary Radford <maradford@ucdavis.edu>, Marcy Uhart <muhart@ucdavis.edu>
Subject: Re: project idea and introduction

Dear Christina,

I would love to discuss more. Thank you and Juan Carlos for reaching out. I have copied Mary Radford who can help us find a time in a couple of weeks to talk. Not sure if you were thinking sooner. The schedule is quite booked, but if you something time sensitive or would like to start up planning for a project, we can try to juggle. I've also copied Marcela Uhart, who is our Latin America Director. It would be nice to include her, as well. In the meantime, I think a collaboration with our developing Global Virome Project (globalviromeproject.org) might be a perfect synergistic fit! Open to other options, too, depending on what you had in mind.

Looking forward to talking.

Have a nice weekend,

Jonna

Jonna AK Mazet, DVM, MPVM, PhD

Professor of Epidemiology & Disease Ecology

Executive Director, One Health Institute

Director, One Health Workforce – Next Generation of USAID Emerging Threats Division

Director Emeritus, PREDICT Project of USAID Emerging Threats Division

Board of Directors, Global Virome Project

School of Veterinary Medicine

University of California, Davis

Institute for Global Health Sciences

University of California, San Francisco

For scheduling and logistical issues, please contact:

Ms. Mary Radford

maradford@ucdavis.edu

On Fri, May 22, 2020 at 1:53 PM Cristina Davis <cedavis@ucdavis.edu> wrote:

Dear Jonna and Christine,

I'm reaching out to tell you about an international effort that I'm involved with that bridges engineering and the biological sciences. Here is a link: https://www.xprize.org/prizes/rainforest

Basically I am a part of a consortia that is trying to field technologies to sequence everything in the amazon (quite a challenge!). There is some planned private philanthropy behind this consortia to fund potential field work and exploratory efforts. My collaborator Juan Carlos is CC'd here.

Our consortia is familiar with your work, and we see a potential intersection of interests. We are wondering if you would be willing to talk and learn more about our effort? If so, we can set up a call for next steps.

Best regards,

Cristina

--

Cristina E. Davis, Ph.D.

Professor and Chair, Department of Mechanical and Aerospace Engineering

Warren and Leta Giedt Endowed Professor

University of California, Davis

Associate Director, UC Davis Clinical and Translational Science Center (CTSC)

a part of the National Institutes of Health (NIH), National Center for Advancing Translational Sciences (NCATS)

TEL +1.530.754.9004

E-mail: cedavis@ucdavis.edu

Website: http://mae.ucdavis.edu/faculty/davisc

KAA INITIATIVE

MEMORANDUM OF UNDERSTANDING

among the Parties of the

KAA INITIATIVE

This Memorandum of Understanding ("MOU") is entered into by and among the Parties set forth in Appendix 1 to this MOU ("Original Parties"), as of the Effective Date specified hereafter. In addition, it is the expectation of those participating in the KAA INITIATIVE ("KAA") that additional Parties from around the world will join the collaboration over time in order to participate in and contribute to the initiative ("Additional Parties") using the process described below. The Additional Parties to the MOU are set forth in Appendix 2 to this MOU. Each of the Original Parties set forth in Appendix 1 to this MOU and each Additional Party that joins the collaboration identified in Appendix 2 to the MOU will be referred to collectively herein as the "Parties".

1. PURPOSE

The purpose of this MOU is to provide a framework for participation in the KAA Initiative objectives as per Section 3 of this MOU through the collaboration of the Original Parties set forth in the Appendix 1 to this MOU, as well as Additional Parties identified in Appendix 2 to the MOU, who will join the collaboration over time. This MOU *does not create any legally binding obligation on the part of the Parties* but the Parties may contemplate entering into future legally binding agreements in the future to further their collaboration. The general intent of this MOU is to establish collaboration plans for the Parties. As Additional Parties join the initiative they will be added to the MOU in accordance with the terms set forth below. The Parties seek to complement each other's respective strengths, experience, knowhow, assets, capabilities and technologies in order to facilitate increased collaboration in the KAA Initiative's science, technology and innovation programs and projects that will be necessary to unleash an inclusive bioeconomy in the Amazon Basin that is in harmony with its peoples and preserves its ecosystems for future generations. Developing autonomous sensing robots for biological discovery of the Amazon at scale can help "write and make sense of the Amazon's Book of Life" providing a science, engineering and innovation foundation for a public-private commons targeted at systems-level change to unleash an inclusive bioeconomy in the Amazon for the benefit of its peoples and its standing forests.

2. BACKGROUND

The KAA Initiative will support the execution of the Earth BioGenome Project (EBP) and Earth Bank of Codes (EBC) partnership. The EBC (www.earthbankofcodes.org) is a public purpose initiative incubated by SpaceTime Ventures that is affiliated to the Center for Global Public Goods of the World Economic Forum in Geneva, Switzerland. The EBC was launched in the World Economic Forum's Annual Meeting in Davos 2018 as a sister initiative of the Earth BioGenome Project (www.earthbiogenome.org) which has a mission to carry out full genome sequencing of all complex life of the planet. Please see https://www.weforum.org/press/2018/01/new-partnership-aims-to-sequence-genomes-of-all-life-on-earth-unlock-nature-s-value-tackle-bio-piracy-and-habitat-loss/ for more details.

In addition, the Rainforest XPrize challenges the Parties to design, develop and test novel autonomous systems technologies, sensor payloads and computational biology innovations necessary to characterize hard to access biodiversity of key terrestrial and aquatic ecosystems of the Amazon (and other Tropical Forests). The Parties of the KAA Initiative are particularly focused on moving the needle to search and fully characterize life of the Amazon Basin– as a key building block in unleashing an inclusive bioeconomy in the Amazon Basin countries. This has become extremely urgent recently in the context of an ever rising risk of being close to transgressing the Amazonian Savannization tipping point with catastrophic consequences for the world at large (https://www.pnas.org/content/113/39/10759).

The Parties recognize that the scope of the KAA Initiative exceeds the capability of any one Party to undertake; each Party recognizes that participating in and contributing to the shared planning, design, development, prototyping, testing and operation of KAA Initiative's science, engineering, innovation and policy-oriented programs, projects and activities makes a small contribution towards saving the Amazon Basin from extinction. As such, the Parties believe that by entering into this MOU they will be able to collaborate in co-developing the programs of the KAA Initiative and further their respective missions in a manner in which they would not be able to do so individually.

Accordingly, the Parties have agreed that a collaborative international, scientific, technological, innovation & policy-oriented effort is needed, and they hereby express their intent to become Parties of the KAA Initiative, and in doing so identify their roles and functions associated with designing and implementing the KAA Initiative.

We the Parties acknowledge that KAA Initiative will conduct its activities in strict alignment with the objectives and principles of the the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity. The Parties acknowledge that the KAA Initiative will have an instrumental role in unleashing a number of inclusive bioeconomy innovations for the benefit of biodiverse nations in the Amazon and other Tropical Forests by supporting an alternative path for economic and social development that preserves life for future generations.

Membership in the KAA Initiative will not affect the normal operations of the Parties.

3. COLLABORATIVE OBJECTIVES OF THE KAA INITIATIVE

At the minimum, the Parties of the KAA Initiative agree to conduct the collaborative science, technology, innovation and policy-oriented activities dedicated to achieving the following goals directly or indirectly:

Development, testing and operation of a family of autonomous aerial, terrestrial, aquatic systems equipped with multiple advanced sensor payloads to *"autonomously sense, predict, and discover life in the Amazon and understand their genomics, the biochemical processes involved, the biological functions at play, their complex interrelationships, the risks of pathogen spillover events leading to future pandemics"* - for future deployment of autonomous sensing robots for biological discovery in the Amazon at scale, so that an inclusive bioeconomy in the Amazon can be unleashed for the benefit their peoples whilst preserving life in the Amazon for future generations.

4. PROGRAM MANAGEMENT AND IMPLEMENTATION OF SPECIFIC PROJECTS

The Parties acknowledge that SpaceTime Ventures will seek to set up a PMO Office and act as a Technical Secretariat for the KAA Initiative focused on orchestration of the various work packages towards achieving the objectives of the KAA Initiative through overall strategy and program planning support for the KAA Initiative, and will act as the institution that will register as "lead organization" with the XPrize Foundation on behalf of the Parties of the KAA Initiative. In due time and with the support of the Parties, a separate public purpose organization will be set up by the Parties (www.kaainitiative.org) to facilitate the governance, grant funding and execution of the program over the next few years.

The Parties will enter into separate written agreements for each specific project arising under this MOU that requires the commitment of funds and other resources from two or more Parties or otherwise necessitates written documents reflecting the Parties' mutual understanding of details related to the planning, development and implementation of the project ("Project Agreements"). Project Agreements involving the exchange of technologies, designs, data or information will include provisions governing intellectual property rights.

5. FUNDING

This MOU creates no financial obligation or firm commitment of staffing or other resources on the part of any Party. It is understood however that all Parties will employ their best efforts to self-fund their own technical staff time for the development of the deliverables associated to the KAA Initiative's "detailed scientific, technology, innovation & policy master planning required to generate the design blueprints and the resource requirements for building and testing autonomous sensing robots for biological discovery in the Amazon in compliance with the Nagoya Protocol and the national laws of Amazonian countries". Execution activities beyond this initial collaborative detailed planning and design effort undertaken pursuant to this MOU shall be subject to base grant funding - *that will be raised by the KAA Initiative and made available to the executing Parties in their own jurisdictions and in particular to those that are carried out in the Amazon Basin* – complemented by additional in-kind resources of each of the Parties. In addition, each Party may need to raise additional funds to execute the research &

development program proposed in this MOU with the formal support and backing of the KAA Initiative. In the case of other international R&D funding opportunities that may appear, it is understood that two or more Parties will negotiate and sign further Project Agreements between themselves to execute additional research & development projects which were not initially planned but that are aligned with the *Collaborative Objectives of KAA Initiative* described in Section 3 of this MOU.

6. AUTHORIZED REPRESENTATIVES

Authorized Representatives of the Original Parties are as shown in Appendix 1, and Authorized Representative of the Additional Parties are shown in Appendix 2 to this MOU.

7. DURATION, TERMINATION AND AMENDMENT

Duration. This MOU shall remain in force for five (5) years from the date it has been signed by at least three Original Parties ("Effective Date"). Any Party may terminate it's own involvement in this MOU by providing 60 days' advance written notice to the other Parties. Separate agreements entered into KAA Initiative's funded projects will identify appropriate mechanisms and timelines for the projects to reach an orderly conclusion in accordance with the relevant Project Agreement.

Extension and Renewal. The Parties may extend or renew this MOU by mutual agreement of a majority of the Original Parties, confirmed in a written amendment signed by each Party's authorized signatory.

Amendment. No amendment of the terms of this MOU will be effective unless made in writing and signed by a majority of the Original Party's authorized signatory followed by required notice of the amendments to all Parties.

Additional Parties. Additional Parties may join the collaboration over time in order to participate in and contribute to the initiative. Additional Parties will be reflected in Appendix 2 to the MOU. They will be bound by all of the terms of the MOU. All Original Parties will be provided written notice that an Additional Party would like to join the MOU. Each Original Party will have fourteen (14) days to raise an objection in writing to the inclusion of a proposed Additional Party to the MOU. If the majority of Original Parties do not object to the inclusion of the proposed Additional Party within this timeframe, the Additional Party will become a Party to the MOU.

Entire Agreement. The terms and conditions herein constitute the entire agreement and understanding by and between the Parties and shall supersede all other communications, negotiations, arrangements, and agreements either oral or written, with respect to the subject matter herein.

8. GENERAL MATTERS

Use of Names. Each Party may use the other Party's name and/or logo in announcing and otherwise promoting this MOU or a specific KAA Initiative Project Agreement, provided that the KAA Initiative is mentioned (together with its standard communication materials) and all language and phrases concerning or referring to the other Party shall be approved in writing in advance by the other Party. Nothing in this MOU shall be deemed confidential.

Nondiscrimination. In the administration of this MOU or any Project Agreements, the Parties shall not discriminate on any basis prohibited by applicable law, including on the basis of race, sex, age, disability, ethnicity, religion, or national origin.

Notices. The Parties must give all notices under this MOU in writing. All communications must be sent to the addresses set forth below or to such other address designated by the Parties by written notice. Notices are effective upon receipt.

Dispute Resolution. The Parties agree to make efforts in good faith to resolve all disputes amicably and expeditiously between themselves by good faith consultation or negotiation by the Institutional Representatives or their designees, or by any other way agreed upon by the Parties.

Non-Binding Nature. This MOU is not intended to and does not give any person who is not a Party to it any rights to enforce any of its provisions. Nothing in this MOU will be construed as creating binding legal obligations between the Parties. This MOU is a broad statement of intent which sets forth the general basis upon which the Parties wish to proceed. No legal liability will arise in respect of any subject matter hereof unless a subsequent binding agreement is negotiated, approved, executed and delivered by the Parties to this MOU. Under no circumstances will this MOU be construed as creating or establishing any formal, legal, association, joint venture, or principal/agent relationship between the Parties.

Force Majeure. The Parties acknowledge that any project arising under this MOU may be suspended or terminated due to an event of force majeure including, but not limited to, fire, earthquake, epidemic, explosion, casualty, strike, act of war, riot, civil disturbance, terrorism, act of God, state, local or national law, decrees or ordinance, or any executive or judicial order, or any other reason beyond the Parties' control. Each Party will notify the other Party as soon as it is aware of any event of force majeure which would delay or prevent the performance. In the event of a force majeure, the Parties will consult with each other to determine a revised timeline for performance or whether to terminate the MOU.

Authorized Signatories. Each Party represents that the individuals signing this MOU have the formal authority to sign on its behalf in the capacity indicated.

9. COUNTERPARTS

This MOU may be executed in any number of counterparts, each of which shall be an original as against the party whose signature appears thereon, but all of which taken together shall constitute but one and the same instrument.

KAA INITIATIVE

Appendix 1:

Original Parties to Memorandum of Understanding on the KAA INITIATIVE

(1) Party name: SpaceTime Ventures

Signed: ______________________________________

Name: Juan Carlos Castilla-Rubio

Title: Chairman

On behalf of: SpaceTime Ventures

(2) Party name: …

Signed: ______________________________________

Name: ______________________________________

Title: ______________________________________

On behalf of: ______________________________________

(3) Party name: …

Signed: ______________________________________

Name: ______________________________________

Title: ______________________________________

On behalf of: ______________________________________

(4) Party name: …..

Signed: ______________________________________

Name: ______________________________________

Title: ______________________________________

On behalf of: ______________________________________

(5) Party name: …..

Signed: ______________________________________

Name: ______________________________________

Title: ______________________________________

On behalf of: ______________________________________

(6) Party name: …

Signed: ______________________________________

Name: ______________________________________

Title: ______________________________________

On behalf of: ______________________________________

(7) Party name: ….

Signed: ______________________________________

Name: ______________________________________

Title: ______________________________________

On behalf of: ______________________________________

(8) Party name: ….

Signed: ______________________________________

Name: ______________________________________

Title: ______________________________________

On behalf of: ______________________________________

(9) Party name: ….

Signed: ______________________________________

Name: ________________________________

Title: ________________________________

On behalf of: ________________________________

(10) Party name: ….

Signed: ________________________________

Name: ________________________________

Title: ________________________________

On behalf of: ________________________________

Appendix 2:

Additional Parties to Memorandum of Understanding on the KAA INITIATIVE

(1) Party name:

Signed: ________________________________

Name: ________________________________

Title: ________________________________

On behalf of: ________________________________

(2) Party name:

Signed: ________________________________

Name: ________________________________

Title: ________________________________

On behalf of: ________________________________

(3) Party name:

Signed: ________________________________

Name: ______________________________________

Title: ______________________________________

On behalf of: ______________________________________

(4) Party name:

Signed: ______________________________________

Name: ______________________________________

Title: ______________________________________

On behalf of: ______________________________________

From: "Oladele Ogunseitan" <oladele.ogunseitan@uci.edu>
Sent: 06/24/2020 4:52:50 PM (-07:00)
To: REDACTED; "Woutrina A Smith" <wasmith@ucdavis.edu>; "Jonna Mazet" <jkmazet@ucdavis.edu>; "Jon Epstein" <epstein@ecohealthalliance.org>; "alexandra zuber" <alexandrazuber@atahealthstrategies.com>; "Omar Romero-hernandez" <oromero@haas.berkeley.edu>; "Carolyn Forlee" <cforlee@ucdavis.edu>
Cc: "onehealthnextgen Sympa List" <onehealthnextgen@ucdavis.edu>
Subject: One Health Workforce Academy & WHO-Academy: Initial Meeting
Attachments: WHO Academy overview v04Jun20.pdf, , Mail Attachment.ics,

Greetings,

I am looking forward to our discussion at 8:00am (Pacific Time) tomorrow Thursday 25 June.

Many thanks to Alexandra for introducing the One Health Workforce team to the WHO Academy team. Thanks also to Maria for sending the zoom link (see below).

We have only 30 minutes for this initial meeting, and we will spend the time productively. Our main goal is to explore potential synergy between the envisioned One Health Workforce Academy (currently being funded by USAID in two regional networks in Africa and Southeast Asia) and WHO-Academy. Both Academies are in planning stages with targets to formally launch publicly at about the same time next year. To guide our discussion, I suggest the following agenda items:

1. Brief introductions

2. Information about the structure, function, and target audience of WHO Academy. I have attached to this email a brochure published in May was shared by David W. Lowrance (WHO) through our colleague Bruce Struminger.

3. Brief description of our vision for the One Health Workforce Academy.

4. Open discussion of convergent goals and aspirations, and opportunities for collaboration.

5. Action items, and plans for follow-up meeting, including Jim Campbell, and others as needed.

Best wishes.

- Dele

WHO ACADEMY

Revolutionizing lifelong learning for health impact

12 May 2020

academy@who.int | #WHOAcademy | academy.who.int

iPhone

Android

Academy COVID-19 app for health workers

WHOA

Health Lifelong Learning Gap

World Health Organization

Current lifelong learning market and capacities do not adequately address competency gaps.

234M jobs in the health economy today, 120M additional created by 2030

1,400+ courses provided by WHO reaching 700,000+ people in 2018

It takes over **10 years** to implement evidence-based guidance

Less than 5% of countries are on track to achieve 11 health targets by 2030

Academy Approach

World Health Organization

The WHO Academy is about more than just building health competencies globally, it's about transforming the way we learn and change behaviors, policies and systems for health impacts.

State of the Art Inputs

Global health expertise & evidence-based guidance

Adult learning science

Hybrid and digital learning technologies

Human performance science & systems thinking

Process

Immersive individual & social learning experience

Quality management & micro-credentials

Research and Innovation

Outcomes

Impact

Behavior

Learning

Engagement

Learning Model

World Health Organization

The WHO Academy learning model applies the best of adult learning science to offer quality multi-lingual lifelong learning tailored to the needs of individuals. Technologies support real-time analytics and feedback, personalization, social collaboration and impact at scale.

Learning analytics, quality assurance, quality control, quality improvement and feedback loops

Hybrid learning approach

Learning experience driven by adult learning science, supported by AI

Social collaboration tools

Digital Learning

Onsite Learning

Advice engine

Complex query search

Personalized immersive learning

Assessments

Micro-credentials

Shared learning and networks

User Experience

World Health Organization

1. **WHO staff member accesses Academy library of courses.**

2. **They enter primary info** *(language, role, region, tech access),* **allowing our advice engine to tailor a custom learning pathway.**

3. **A complex query engine allows them to find detailed answers at any time.**

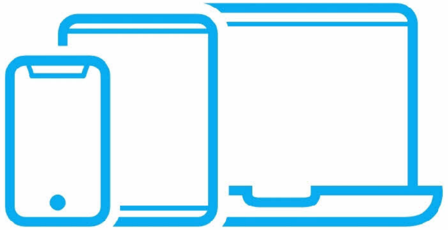
4. **They are connected to peers working in similar roles across the globe.**

5. **They access digital/onsite learning experiences** *(videos, assessments, games, and exercises)* **to support their immersive learning.**

6. **They then complete a final assessment to receive a digital credential.**

Learning Modalities

World Health Organization



Digital learning

Accessible via laptop, mobile phone or tablet and once downloaded, can be used offline.

May 2020: Academy app

Multilingual one-stop-shop for health workers on COVID-19: technical resources, training, virtual classroom environment, peer learning

Onsite learning

Onsite immersive learning experiences such as cutting-edge health emergency simulations will be facilitated at the WHO Academy campus network including a new Hub in Lyon, France and regional spokes.

Portable learning lab

Designed for contexts where participants cannot travel or access the digital learning platform, such as in health emergencies and hard-to-reach areas with limited connectivity.

Target Reach & Audience

World Health Organization

WHO Academy aims to target 10 million people by 2023

234 million health workers globally

$6 billion spent by Official Development Assistance on training and capacity building

$30 billion spent on lifelong learning in health *(2025)*

The Academy will support four major roles—any user could hold multiple roles in different areas of competency

Roles	Description	Goals
Leading	Formulating, coordinating, managing or steering the implementation of policy and/or practice.	Decisions result in the most effective, efficient, sustainable and impactful policy and/or practice to resolve a problem or challenge in their context.
Practicing	People engaged in an occupation that contributes towards better health outcomes	Develop and maintain competencies in actions that result in a desired outcome or impact, enhance professional satisfaction, career development and opportunities.
Catalyzing	WHO staff, consultants across all grades, roles and levels of the organization. UN staff.	Support range of stakeholders to take and/or implement better decisions for greatest impact.
Staying healthy	People responsible for their own health and that of others.	Engage in or take decisions that result in the best health outcomes for themselves and that of others.

How We Differ

1. **A single platform** for learning, globally accessible, and offline capable—perfect for remote areas

2. Targeted and **tailored multilingual learning** for individuals and teams, **customizable** to user needs

3. **Measurable impact** based on outcomes and learning **analytics**, adapting courses to improve over time

4. Accredited courses ensure quality with **verifiable credentials**

5. Co-created courses **built in tandem** with users based on specific needs

6. Learning **built to scale**—WHO reach can ensure global access for millions of users

UCDUSR0013402

Set-up Phase Workstreams:

Launch May 2021

1. STRATEGIC MANAGEMENT

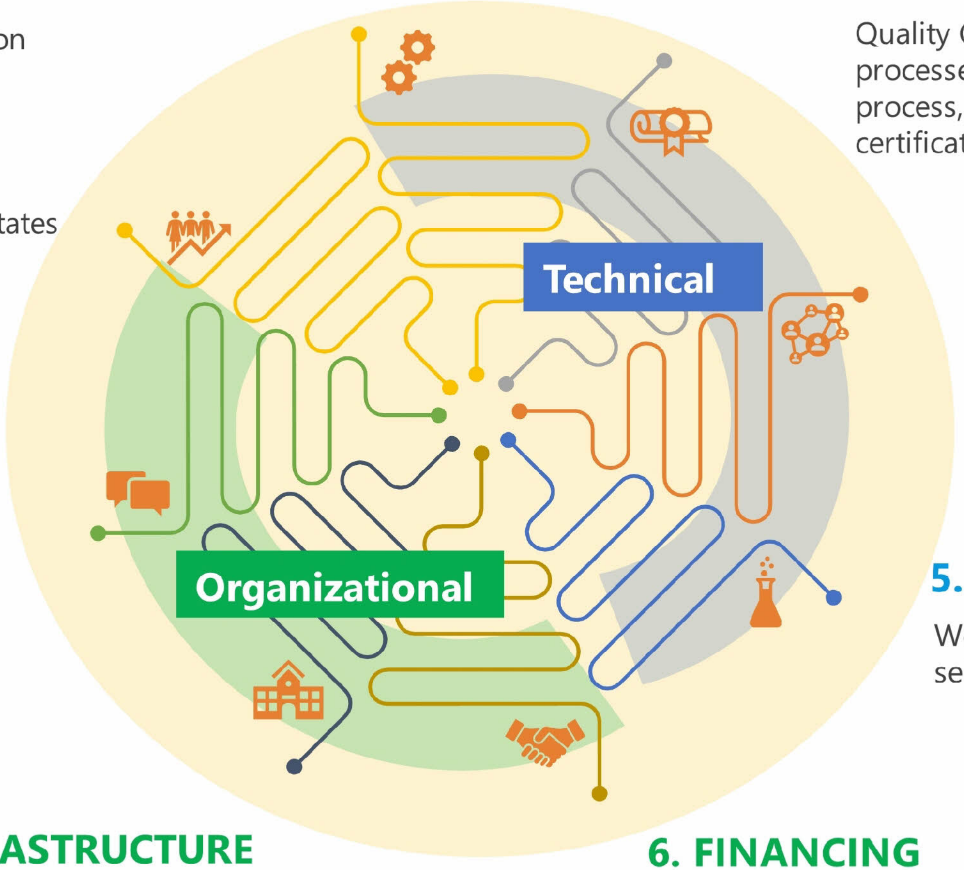
Strategic development, implementation coordination, project management, resource management, institutional transformation, governance, strategic partnerships and internal/external stakeholder management, Member States engagement (cross-cutting)

2. LEARNING STRATEGY

WHO learning strategy development, consultation and adoption

3. QUALITY

Quality Committee, standards, processes, procedures, accreditation process, micro-credentialing, digital certification



4. LEARNING TECHNOLOGIES

Learning experience platform and technologies development, AI engines, integration with library services, learning model application through prototypes

5. COURSES

Working prototypes, course selection for 2020, evaluation

6. FINANCING

Financing strategy, cost recovery model, financial sustainability

7. INFRASTRUCTURE

Design brief, infrastructure specifications, strategy and plan

8. COMMUNICATIONS, ENGAGEMENT AND MARKETING

Stakeholder engagement, communications and social marketing strategy development and implementation

Establishment Blueprint

World Health Organization

3 prototypes	10 courses			30 courses	60 courses	100 courses	140 courses	180 courses
Prototype	**Beta-test**	**Evolve**	**Launch**	**Improve**	**Scale**	**Establish**	**Expand**	**Sustain**
July 2019 – June 2020	July – December 2020	January – April 2021	May 2021	June – December 2021	January – December 2022	January – December 2023	January – December 2024	January - December 2025
Ideate and test prototypes, standards and systems	Develop and test courses, standards and systems	Review and refine courses, standards and systems	Live launch of the first10 digital courses	Real-time analytics, tests and quality improvement	Build coverage across target audiences in all regions	Establish hub and spokes campuses and systems	Scale operations, systems and coverage	Achieve financial self-sustainability

1. Set-up (2019-2021)

Creating and transforming internal structures, capacity, platform, courses and systems. Agile approach. Consultation on strategies and standards. Building networks and stakeholder engagement. Lyon Hub infrastructure development.

2. Start-up (2021-2022)

Consolidating the operational model. Operationalising course development, delivery and quality improvement systems. Lyon hub infrastructure development.

3. Scale-up (2023-2025)

Operationalising Lyon Hub and regional spoke infrastructure and systems. Expanding operational systems for targeted scale and sustainability.

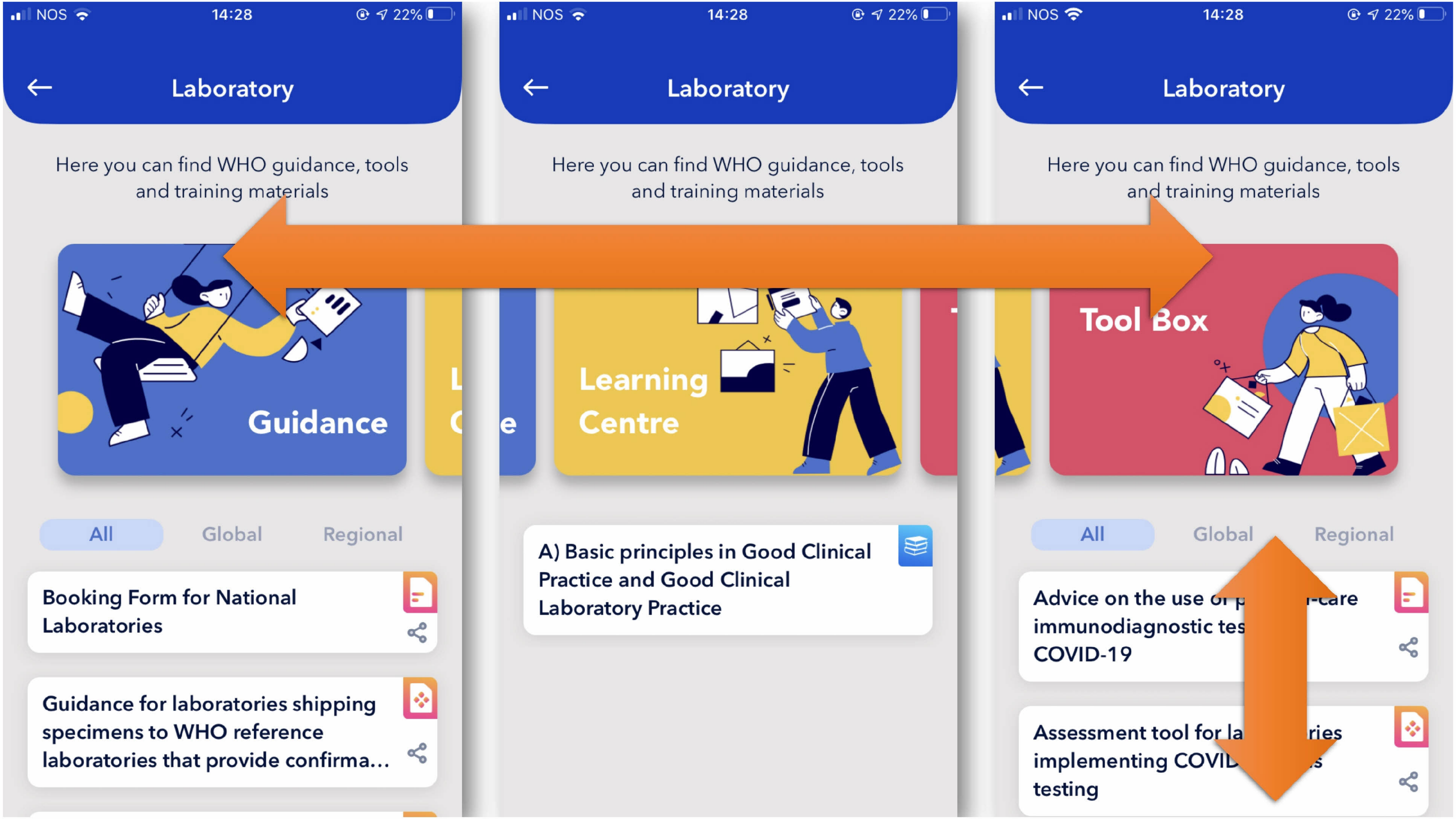
WHO Academy app

- IOS and Android
- 7 Languages
- More than 1.000 links
- WHO's COVID-19 Database search
- Daily updates
- Almost 40.000 downloads
- 205 countries and territories

Next release: 10.06.20 (Sprint 2)

- Push notifications
  - new features
  - new contents
- Personal bookmarks and offline content
- Improved search capability
  - Events, literature, news…
- Sitefinity API for News


NOS 14:27 22%
Learn about COVID-19
Search for resources
What would you like to learn?
Case Management
How to manage care for COVID-19 patients
News
Events
Literature
General
Settings
NOS 14:27 22%
Learn about COVID-19
Search for resources
What would you like to learn?
IPC & Occupational Health
Protecting health workers and the community
News
Events
Literature
General
Settings
NOS 14:27 22%
Learn about COVID-19
Search for resources
What would you like to learn?
Epidemiology
Distribution, characteristics and determinants of COVID-19
News
Events
Literature
General
Settings



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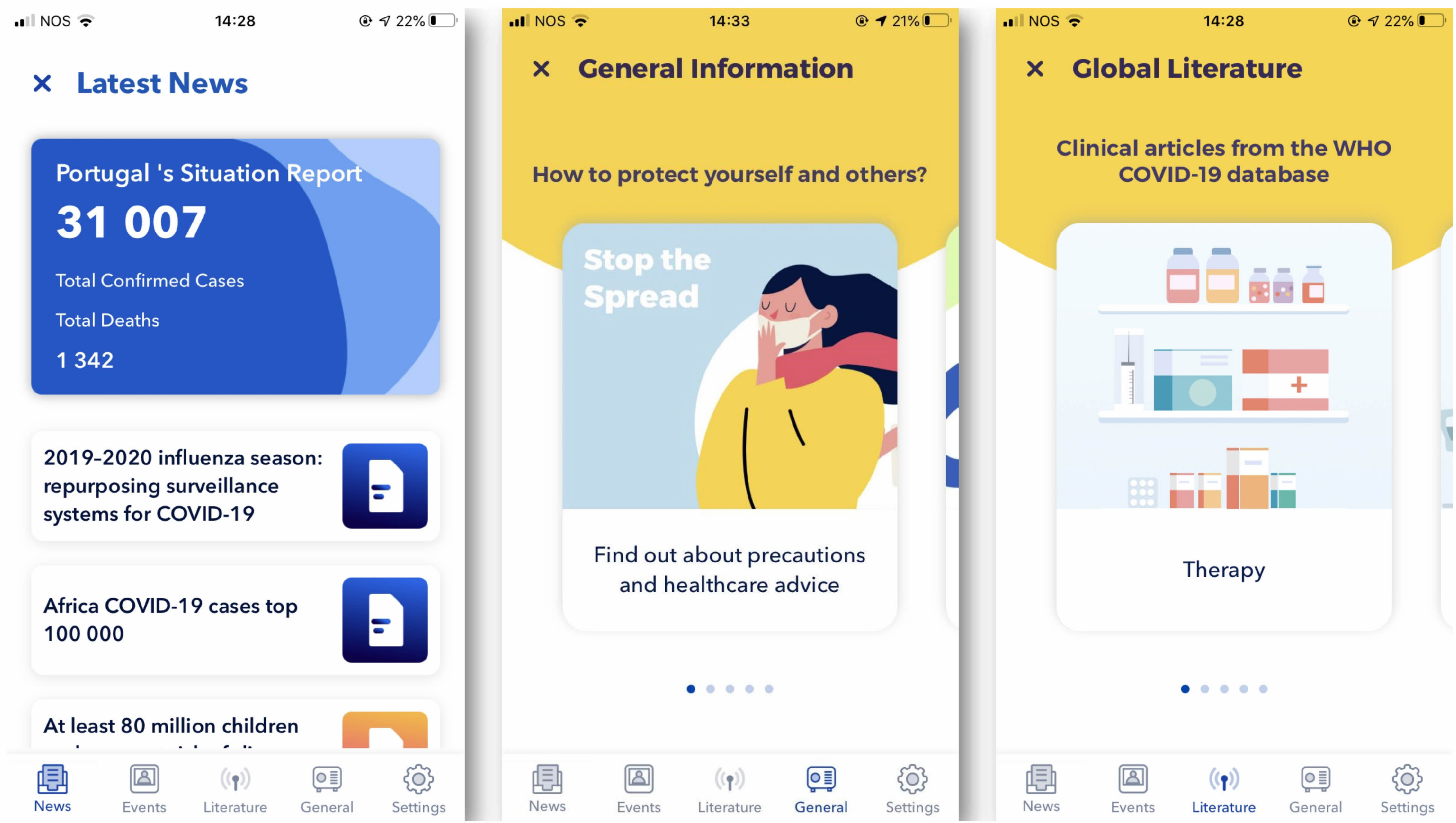

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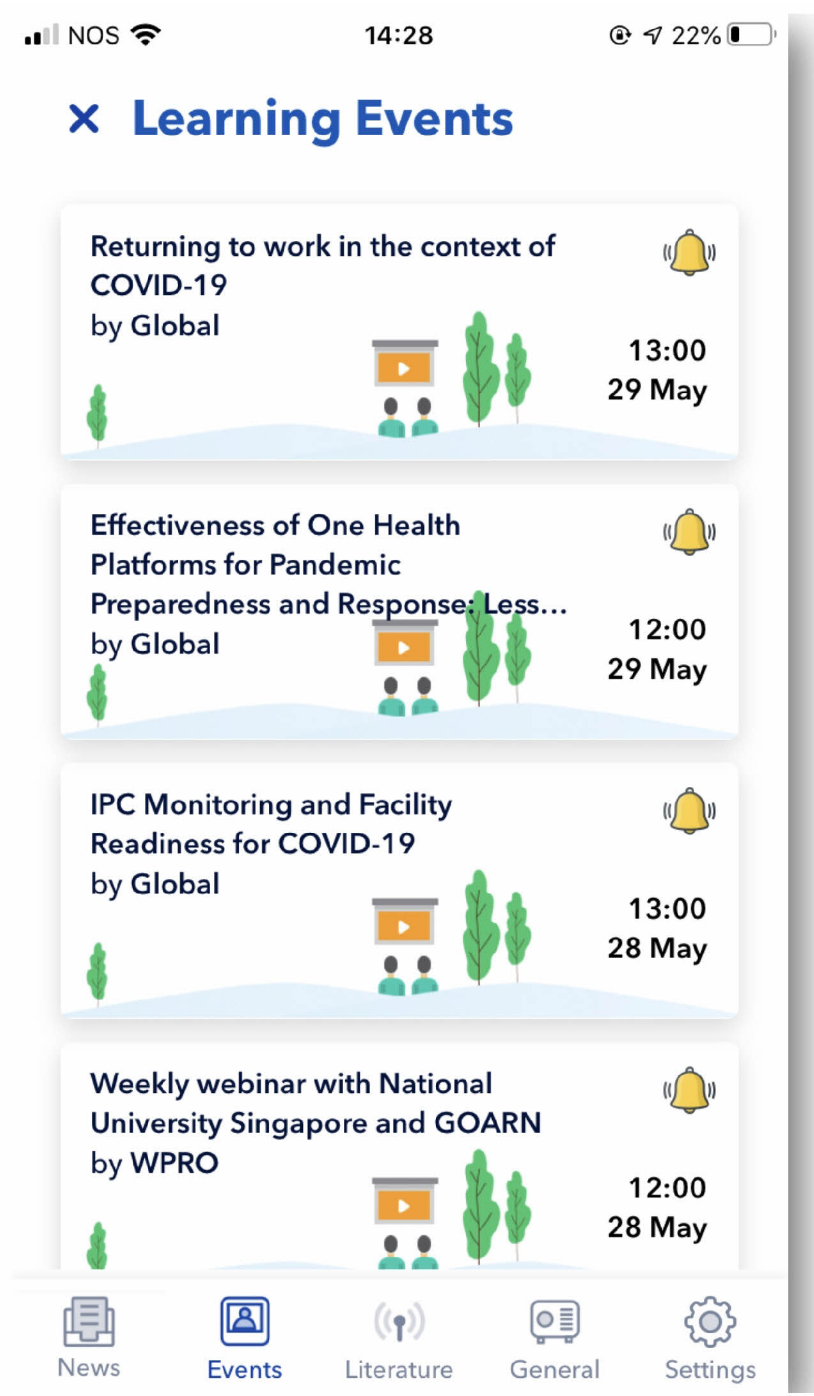

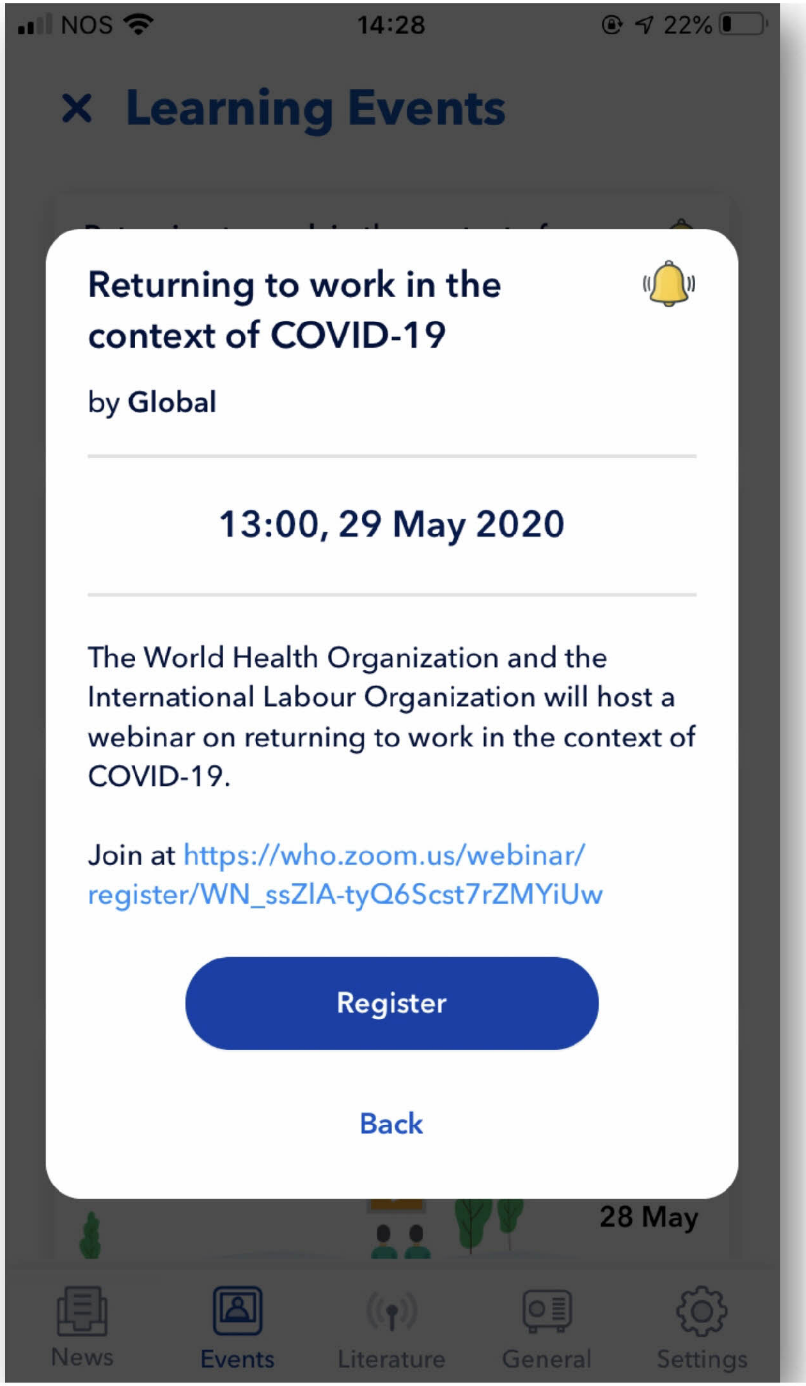

- **Register for future events**
- **Join live events**
- **View past recordings**

THE FUTURE

- COVID-19
- Ebola
- Cholera
- Diphteria
- Polio
- …

THE FUTURE IS HERE

To develop: “Academy style”, state of the art, mixed reality, competency based courses, linked to a digital credential system

Questions on applications for development of Academy courses

Dena
REDACTED

Questions on WHOA app contents and webinars

Ave
REDACTED