A. Overview

Pathogens transmitted by arthropod and bat vectors continue to burden the health of humans around the world. Malaria is perhaps the most deadly infection in the tropics. West Nile, chikungunya and Zika viruses have emerged as major global pathogens. Tick-transmitted infections such as Lyme disease and Powassan virus continue to emerge in temperate regions; and the bat-associated ebolaviruses, Nipah virus, rabies virus and others are among the most dreaded infections known. Agents vectored by arthropods and/or bats thus constitute some of the most feared, difficult and persistent problems affecting human health.

Colorado State University (CSU) established the Arthropod-borne and Infectious Disease Laboratory (AIDL) as a visionary approach to counter these emerging threats. Since its creation in 1984, AIDL has been an internationally recognized resource advancing science, practice and training on topics related to vector borne infectious disease. One of the many unique aspects of AIDL includes housing one of the only captive breeding colonies of bats for use in infectious disease research, and BSL2 and BSL3 insectaries. After 35 years of use, AIDL facilities at CSU are in need of significant enhancements-- roofs and walls leak during inclement weather, mold has been a problem in insectaries, buildings have had issues with vermin control, and temperature is poorly regulated. These issues have placed our unique and productive research and training programs at risk.

Recognizing this, Colorado State University (CSU) has committed $22M to construct a new building, the Center for Vector-Borne Infectious Diseases (CVID), to replace aging AIDL infrastructure. CVID construction is scheduled to begin in mid-2019. While CSU's commitment of $22M is laudable, it is insufficient to complete the full suite of projects envisioned to provide specialized, state-of-the-art research space to support AIDL's research priorities for the next 35 years. This proposal thus outlines requests for funds to enhance planned CVID construction. The goals of this proposal are to:

1) Construct a state-of-the-art insectary with the necessary environmental and biosafety controls capable of accommodating our diverse research needs in the area of arthropod-borne diseases
2) Construct bat housing to accommodate a growing research agenda and national need in emerging bat-borne and bat-associated diseases
3) Enhance the CVID through improved tissue culture, gas control, autoclave and elevator equipment

Our approach to accomplish these goals is as follows:

1) Construction of a 3,853 SF insectary. This will include 11 temperature, photoperiod and humidity controlled environmental chambers to house arthropod colonies. One large (385 SF) chamber will provide the capacity to conduct population cage studies. We will install a wind tunnel for studies of indoor insecticides. This space will include three workrooms, and a closed manipulation chamber.
2) Construction of a 1,070 SF bat facility. This will include flight rooms to maintain a breeding colony, and rooms to hold bats under ABSL-2 containment, a manipulation area, and spaces for donning and doffing of personal protective equipment (PPE).
3) Enhance the CVID facility functionality and biosecurity via inclusion of an elevator to support animal transport, 3 autoclaves and enhanced tissue culture capabilities.

The proposed building will support $1.7M in NIH funded research (total direct in FY19) and $4.0M dollars of total sponsored research in FY19. In addition to supporting ongoing AIDL research, several university-wide initiatives, including the CSU One Health Institute and Infectious Disease Research and Response Network, will benefit from enhanced facilities for arthropod and bat work. The CVID also will support the regional community by providing needed laboratory space for a nascent "Front Range Arbovirus Consortium" that is forming in partnership with UC Denver and the University of Northern Colorado. Proposed research facilities for arthropods and bats will be the only one in our region that are co-housed in an academic setting. Our proposed project will greatly enhance planned new construction and create a unique-in-the region research and training resource that will have a direct and sustained impact on research at CSU and globally.

B. Scientific Justification

Goal 1. Construct a state-of-the-art insectary with the necessary environmental and biosafety controls capable of accommodating our diverse research needs in the area of arthropod-borne diseases

Institutional Commitment. A strong focus on vector-borne infectious diseases has been a continuing hallmark of the CSU research portfolio since the founding of AIDL in 1984. Since its creation, the AIDL has been an internationally-recognized resource advancing science, practice and training on topics related to vector borne infectious disease. These include foundational work in the field: mosquitoes genetically engineered to be resistant to arboviruses and to pass on dominant lethal traits(*1-5*); anti-viral RNA interference in mosquitoes and ticks(*6-10*); insecticide control of vectors and resistance genetics (*11, 12*); arbovirus evolution, diversity and recombination in vectors (*13-18*); and critical studies of Zika virus transmission by mosquitoes and the sexual route (*19-23*). The work of the AIDL has also contributed key molecular tools to study virus biology (*24-28*), low-cost diagnostics for use in developing countries (*26, 29-31*), and developed low-cost, non-toxic drugs (Ivermectin and derivatives) to mitigate malaria and arbovirus transmission and disease (*32*). The AIDL at CSU currently includes nine faculty members with expertise ranging from metabolism and biochemistry to arbovirology, entomology and bat immunology. Five of these were hired into tenure track positions within the past six years. To further enhance our work, CSU has committed $22M to construct a new building to house these faculty, who are currently spread among five buildings on all three CSU campuses. Moreover, CSU has displayed a strong institutional commitment to research on arthropod-borne diseases that clearly remains extremely strong.

Infrastructure deficiencies. Despite the research excellence of the AIDL, several infrastructure deficiencies impede our research progress. The first of these is that researchers with a shared focus on arthropod-borne infections are dispersed. The majority are housed three different buildings at the CSU foothills campus, the site of the proposed CVID. Others maintain labs on the main campus and at the Veterinary Teaching hospital to the south of the main campus. This physical separation is problematic because it makes it difficult to leverage shared equipment and technical expertise, and to frequently discuss research results. Thus, the benefits of collaboration and team science are not maximized. A second major problem with the infrastructure is that several of our buildings are well past their useful lives. They leak when it rains and are rapidly degrading (**Figure 1**). In addition, their design is neither suitable for high-humidity insectaries nor up to current standards for working environments. Insectaries are frequently moldy and many labs and offices are located in windowless basements. The basement location of the labs coupled with the leakiness of the buildings has resulted in several incidents in which valuable equipment was nearly damaged by water entering via the ceilings or under walls. The current infrastructure supporting vector-borne disease research is thus deficient in that it hinders collaboration, is badly degraded and poorly suited to its current use.

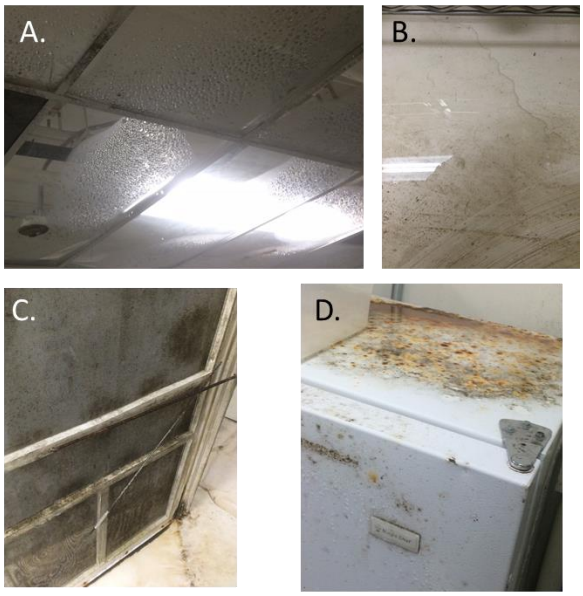


Figure 1. Infrastructure deficiencies result in condensation on the ceiling of existing insectaries (A.) which leads to precipitation that collects on the floors (B.), collectively contributing to mold, mildew and other equipment failures (C, D).

Needs of the community. The community of researchers and trainees at CSU who focus on vector-borne diseases is well-funded and highly collaborative but scattered across various campuses in deficient facilities. Relocation of laboratories to a central state-of-the-art facilities will ensure continued excellence in vector-borne disease research. Several key NIH-funded projects will be directly enhanced by construction of a new insectary co-located with our BSL-2 laboratories and offices (See attachment, Table 3). The following projects, for example, will be ongoing when the proposed project is completed, and will thus be directly impacted by this proposed project:

- Managing insecticide resistance in *Aedes aegypti* mosquitoes (Black WC, R01 AI121211)
- Evaluating the links between arbovirus ecology and evolution (Ebel GD, R01 AO067380)
- Engineering virus resistance into vector mosquitoes (Olson KE, R01 AI130085)
- Using Ivermectin to control malaria (Foy BD, U01 AI138910)
- Assessing scale in vector-borne pathogen transmission among ruminants (Mayo C, 2019-67015-28982)
- Bioassays for pathogen and insecticide activity in vectors (Foy BD, NIH IDIQ 75N93019D00006)

Additionally, nine other faculty are currently funded and/or have applications in review or in preparation for spring 2019 deadlines. This project will thus support $24.5M additional dollars in pending support (submitted as of 2/28/2019). The direct benefit of CVID facility enhancements to these researchers is significant and clear.

In addition to these direct benefit, the proposed project will have a broader impact on the CSU campus through linkages to the CSU One Health Institute (OHI) and the Infectious Disease Research and Response Network (IDR2N). Currently the OHI is heavily invested in research on vector-borne disease and has supported a project using mosquitoes as disease surveillance devices in Guatemala (Ebel GD, PI). The IDR2N hosts annual meetings to discuss current topics such as metabolism (https://metabolism.idrtalks.colostate.edu/) and advanced diagnostics (planned for 2019). Additionally, several non-AIDL affiliated CSU faculty work with viruses and collaborate frequently with investigators who will occupy the CVID. The proposed insectary enhancement of the CVID will therefore create ripples across campus that will meet diverse research needs of others at CSU.

Finally, the CVID insectaries will meet the needs of a broader, regional community. Most significantly, the CSU AIDL is partnering with the University of Colorado (CU) and the University of Northern Colorado (UNC) to launch an Arbovirus Research Consortium. This consortium will bring together expertise in large scale clinical studies, field sites in Africa, Asia and South and Central America, and expertise in virus ecology, evolution and pathogenesis. This consortium formalizes ongoing partnerships between CSU and CU researchers who collaborate frequently, both informally and through joint grant applications (see attached letters of support). For example, the PI of this application, Dr. Ebel, currently collaborates with Dr. Tem Morrison (CU) on a chikungunya virus project. Dr. Morrison's studies have identified mutations to the virus genome that attenuate pathogenesis in mice. Dr. Ebel has the ability to assess the impacts of these same mutations during mosquito infection. Importantly, the enhanced facilities at CSU proposed here will permit a significant expansion of these types of collaborative studies and encourage a more collaborative consortium to develop. We similarly anticipate that additional collaborations with CDC, located in close proximity to the CVID and international partners will also be enhanced. *Given the unique capacity of CVID laboratories that will be afforded by the enhancements outlined in this proposal, the impact of this project is expected to extend beyond the immediate needs of researchers at CSU.*

Goal 2. Construct a state-of-the-art biosafety level-2 bat infection suite

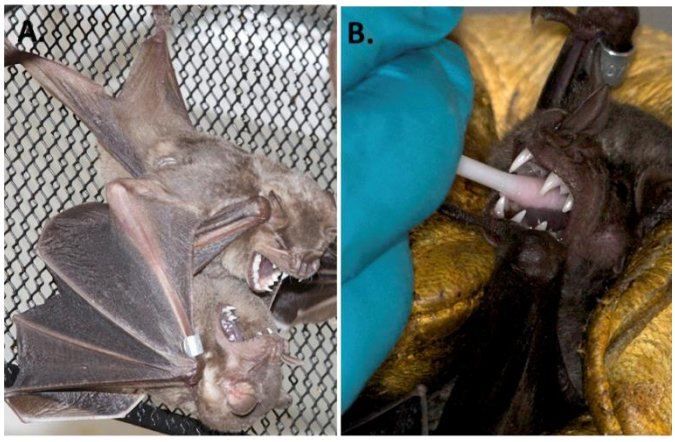


Figure 2. *Artibeus jamaicensis* in the CSU colony (A) and undergoing oral swab (B).

Institutional Commitment. CSU also has one of only two US-based captive breeding colonies of bats for the study of infectious diseases. (The other is housed at the CDC Headquarters in Atlanta.) This colony was established at CSU in 2015 with Jamaican fruit bats (*Artibeus jamaicensis*), which are among the most common and largest bats in the New World, and found as far north as the Florida Keys. Initial funding for this colony was provided by an NIH-supported project (Emerging Virus Disease Unit) to examine zoonotic virus transmission from bats. Bats are known or suspected reservoir hosts of many important emerging zoonotic viruses, including ebolaviruses, Marburg virus, SARS and MERS coronaviruses, Nipah and Hendra

henipaviruses, and rabies virus and other lyssaviruses (*33*). CSU has provided substantial institutional support for our colony, including renovation of facilities to accommodate the special needs of bats and providing staff funding and training. Our current colony room is approximately 4 x 6m and its walls, floors and ceilings are coated with epoxy resin to allow power washing of bat guano twice per week. Landscape fabric is draped on the walls and across the interior to provide hiding substrates for the bats, and skewers to hang fruit are on the walls to facilitate foraging behavior and provide enrichment. The initial breeding colony of 39 bats has expanded and we currently have 150 bats in the colony. The proposed space addition will allow up to 300 bats to be housed in the colony. CSU has thus demonstrated a strong institutional commitment to research on bat-associated emerging viruses by supporting the creation of our current facility.

Infrastructure deficiencies. While our bat breeding colony has been highly successful, the significant increase in demand for bats as highly relevant animal models has outstripped our ability to increase colony size within the context of our current infrastructure. One of our most significant challenges is meeting the requirement for flight rooms. Bats housed in cages that do not permit normal flight can develop stiff joints, *Pseudomonas* septicemia, and pneumonia associated with a lack of normal flight behavior or exercise that can be fatal (*34*). We have developed "walk-in" cages that allow the bats to fly during experiments; however, lack of space in our current animal facility has limited our ability to house multiple experimental groups simultaneously. To circumvent this issue, the proposed CVID bat area will have two ABSL-2 flight containment rooms that will permit us to conduct concurrent infection experiments. The manipulation room will have equipment for necropsy, including a biosafety cabinet, and a foyer for donning and doffing PPE, providing necessary biosecurity controls and preparing food. These proposed improvements to our infrastructure will correct existing deficiencies and permit us to conduct experiments involving bats that support physiological needs and health of animal colonies.

Needs of the community. Construction of the proposed bat facility will help meet the immediate needs of the CSU research community. Current projects that will be impacted include:

- Investigating a novel HL18NL11 bat influenza A virus (Schountz, R01 AI134768)
- Middle East respiratory syndrome coronavirus (MERS-CoV) (Schountz, Subcontract PI R01AI140442)
- Zika virus infection [See Ref (*35*)]
- Cedar henipavirus (Grant pending)

Beyond investigators at CSU, this project will enhance regional and national efforts to understand the complex role of bats in virus emergence (See attached letter of support from Dr. Heinz Feldman, NIH Rocky Mountain Laboratories). For example, bats from our colony have been used to study virus-host associations of Tacaribe arenavirus (*36, 37*) and MERS-CoV (*38*) as *in vivo* models. Studies planned with collaborators at Rocky Mountain Laboratories in Hamilton MT will assess the susceptibility of bats to Nipah virus and ebolaviruses. The colony is also a valuable source of tissues and reagents for in vitro studies. For example, samples from our animals have been used to generate bat cells to study ebolavirus infection (*39*). Cells derived from our colony have been provided to eight collaborators through the US and globally. We have generated over 100 gb of transcriptome data, monoclonal antibodies to bat IgG and IgA, and recombinant bat cytokines that have been provided to the greater scientific community. Moreover, we are currently generating publicly available data and reagents that will meet the needs of the broader community of researchers interested in bat-associated viruses. *Thus, this colony serves as a unique emerging national resource for studies of diseases of bat origin.*

Goal 3. Enhance the CVID design through improved gas control, autoclave and elevator equipment

Institutional Commitment. As noted previously, CSU has generously committed $22M to construct the CVID. While substantial, to fall within this budget and meet our needs of accommodating all AIDL laboratories at one site, we have made compromises to building design, engineering and equipment. We have reduced overall costs through value engineering of HVAC systems, repurposing of old pieces of equipment and making sacrifices in accessibility and convenience that were deemed expendable. A major goal of this application, therefore, is to provide funds to leverage CSU's commitment to our research and reintroduce new equipment and functionalities to the CVID. In particular, we will build tissue culture suites and furnish them with biosafety

cabinets, install a large CO2 manifold system to support our tissue culture suites, purchase new autoclaves for the building and construct an elevator to connect the CVID with the ground floors of adjacent buildings. Moreover, this goal leverages a strong institutional commitment from CSU.

Infrastructure deficiencies and needs of the community. Our overall infrastructure is currently deficient (see above sections for Goals 1 and 2). **Goal 3** seeks to improve the current plans for the CVID to minimize deficiencies built into the design. For example, significant portions of funded research are focused on primary cells and samples from bats and/or arthropods. Currently samples must be transported to different buildings prior to use. Locating tissue culture facilities in close proximity to our insectary and bat facilities is a key enhancement to the CVID. Additionally, our current plan is to move existing autoclaves from our currently-occupied buildings into the new CVID. These autoclaves frequently malfunction and there is a legitimate concern that they will continue to do so in the CVID. Hence our request to purchase new units. Similarly, while CO2 tanks and manifolds can be stored at the point of use, within a tissue culture suite, maintaining CO2 levels and restocking tanks, etc., is more efficient and effective using a centralized system, keeping tanks out of the lab space. We thus propose to keep all gases and a manifold system in a readily accessible part of the CVID under unified control. Finally, due to the contour of the land upon which the CVID will sit, it will be lower than the other buildings against which it abuts. To provide connectivity and ease of transporting research materials from the CVID into our BSL3 insectaries and animal facilities, we would like to construct an elevator. These requested changes will correct current infrastructure deficiencies and increase the efficiency of the CVID.

Long-term vision and institutional support.

CSU established itself as a center for research on emerging and arthropod-borne infectious diseases in the mid 1980s, and has invested significantly in faculty, infrastructure and resources to maintain its position as an international leader in combatting vector borne and emerging viral diseases. As noted, $22M has been committed from institutional funds to make a significant effort in replacing antiquated facilities; this application requests funds for additional enhancements that will truly complete a state-of-the-art center.

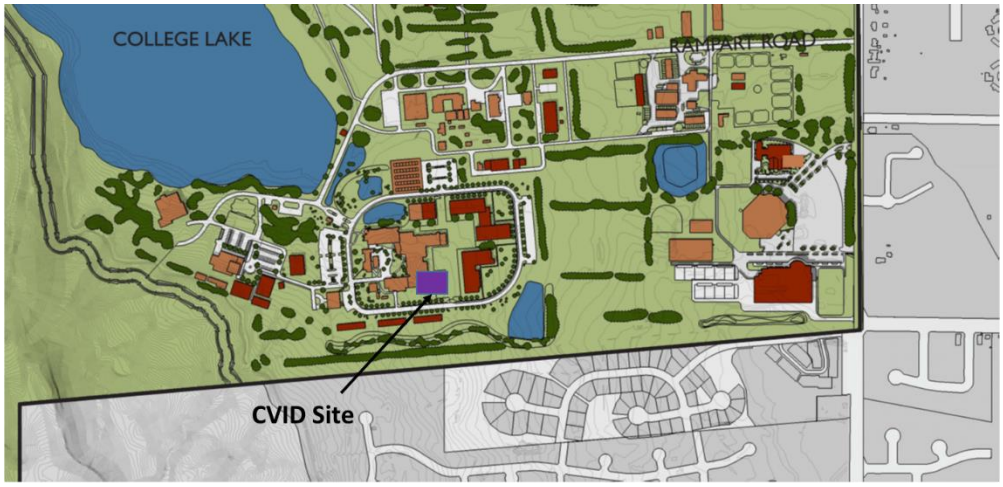


Figure 3. Detail of "Foothills Master Plan, 2015." Existing buildings shown in orange, planned construction shown in red.

To accommodate the ongoing growth the population of Colorado and concomitant expansion of the CSU educational and research mission, the CSU vice president for research, Dr. Alan Rudolph, has commissioned a Foothills Visioning team to help guide the inevitable growth of the foothills campus (where the CVID will be located) to continue to evolve this site into a vibrant, integrated academic campus. This team meets on an ongoing basis to assess issues of traffic flow, parking, services and construction. A master plan **(Figure 3)** that was updated in 2015 is serving as a guide to our ongoing discussions about how best to use foothills campus to accommodate the growth of CSU. *The CVID building is one of the first implementations of the master plan due to the robustness of the research programs it will house and its proximity to CDC and USDA facilities in Ft. Collins and to other key CSU groups such as the Center for Atmospheric Science.*

Organizational and communications plan during the CVID planning and execution.

The planning for this facility began in 2017 and included multiple stakeholder meetings to get to the final design. The space included in this proposal was part of those discussions. Once the project begins, the PI will

communicate weekly with Co-Investigators Lock, Kendall, Foy and Schountz via teleconference and conduct face-to-face meetings at least monthly during the project period.

C. Development of the Facility

Introduction: The new CVID building will be constructed on the southeast corner of the Judson Harper research complex at the CSU foothills campus (see **line drawing 2**) with direct connections to the existing Regional Biocontainment Laboratory (RBL) and Biomedical Research Building (BRB, **line drawing 2**). The areas of the CVID to be completed through this proposed project are located at the northern end of the building, and are shaded in **line drawing 3** (blue = bat area, green = insectary, yellow = autoclave/glasswash, red = tissue culture, orange = elevator). Additional line drawings provide detail for each of these affected spaces. Egress from the building is indicated in **line drawing 4**.

MEP and fire protection summary: Area-specific details are provided in specific sections, below. The insectary, the bat rooms and the tissue culture suites will be served by two redundant 100% outdoor air AHUs (Air Handling Units) and two redundant Energy Recovery Units (ERUs, exhaust fans with energy recovery features). Insectary and tissue culture suites ventilation rate is 8 air changes per hour during occupied hours and 4 air changes per hour during unoccupied hours. Bat rooms ventilation rate is 15 air changes per hour during occupied hours and 4 air changes per hour during unoccupied hours. Ventilation rates and occupancy hours are user adjustable. All mechanical equipment serving the insectary, bat rooms and tissue culture suites is located outside the spaces in the mechanical penthouse for easy maintenance access. Detailed HVAC plans for the proposed CVID are provided in **line drawings 10 and 11**.

These rooms will be provided with handwash sinks with hot and cold water connections. Bat area will be provided with hose reel and trench drains for washdown purposes. Detailed plumbing plans are provided in **line drawings 16 and 17.**

Direct Digital Controls (DDC) system is provided for mechanical equipment controls and monitoring. Airflow control devices are provided to these spaces for directional airflow control. Air flows from “cleaner” spaces to more contaminated spaces.

Autoclaves will be provided with medium pressure steam for decontamination and floor drains for waste discharge. Central steam system is also used for building heating and domestic water heating.

The entire building is protected with a wet-pipe sprinkler system. Fire water main will be connected to an existing fire water main near the building on the CSU Foothill Campus. A detailed fire alarm plan is provided in **line drawing 14**.

Electrical, lighting and security summary: The CVID facility will be provided with a standby generator to support critical systems and equipment in insectaries, bat rooms, and tissue culture suites. The critical loads shall consist of essential HVAC equipment and controls, laboratory equipment, emergency lighting, and special low-voltage systems being supported by the standby generator. Branch circuit panelboards serving insectary, bat areas, and tissue culture suites will be located outside of the respective areas and separate from other panels serving general administrative areas.

Raceways and device boxes for all electrical systems in insectaries and bat rooms will be sealed to prevent vermin harborage and passage through raceways. Boxes will be cast, FD type boxes with neoprene gaskets. Raceways will be sealed at the box with RTV silicone after conductors have been pulled into the respective boxes. Electrical and power plans are provided in **line drawings 12 and 13**.

Sealed and gasketed LED lighting fixtures with IP65 rating and UL listed for wet locations will be utilized in insectary and bat rooms. Tissue culture suites will be provided with standard lensed, recessed LED fixtures. Automatic diurnal lighting control with local override switching will be provided for bat rooms. Lighting levels in each space are based on the current NIH DRM standards.

Security camera and access control system will be provided to monitor and limit access to secure areas including entry to the facility, bat rooms, insectary, and tissue culture areas. Data outlets will be installed for all user workstations and designated locations for remote monitoring of sensitive equipment. The CVID fire alarm

system will include automatic and manual initiation devices with audible and visual notification throughout the building. Telecommunications plans are provided in **line drawing 15.**

Quality of life summary: The CVID has been designed specifically to improve the corridor width and the quality of life of workers. However, the spaces included in this project request require containment and a high level of temperature, humidity and access control. Therefore, compared to other spaces in the CVID, they tend to minimize natural light. They are, however, designed to optimize ergonomics and workspace efficiency.

C.1 Goal 1. Insectary.

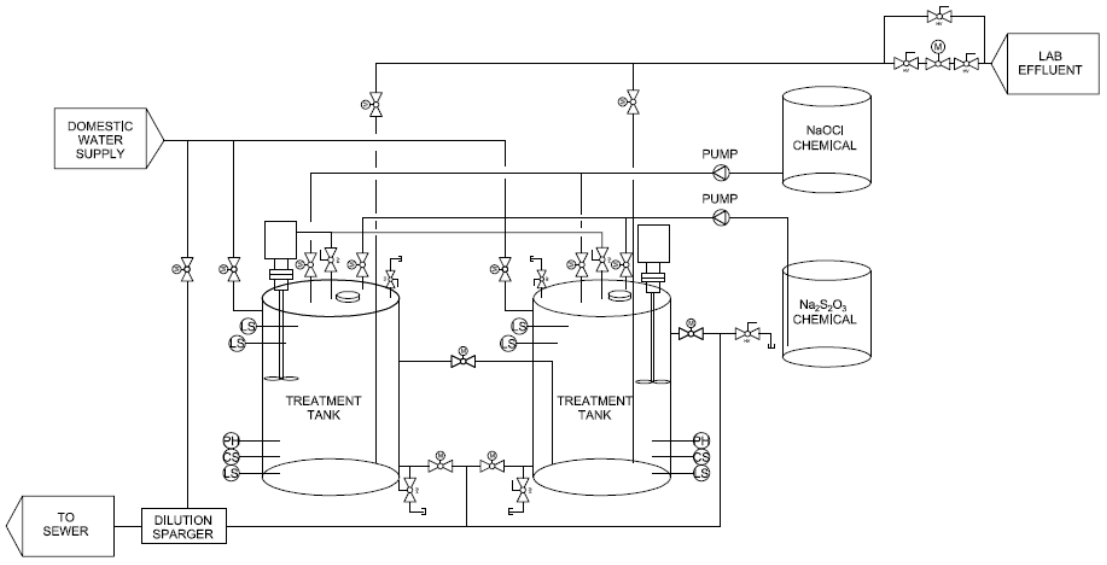
The insectary that will be part of the CVID will be a total of 3853 SF (**line drawing 5**). The walls and ceilings will be composed of smooth, reinforced fiberglass panels to facilitate sanitation. Joints between panels will be sealed with architectural urethan sealant (or 100% silicaone ASTM C920) to make them impervious to moisture. The floor will be slip resistant and coated with an anti-skid additive to the epoxy coated finish. The epoxy coat will have a 6" lap over the wall to create a transition that will prevent leakage and facilitate sanitation. Both the floors and the walls will be white so that insects are visible if there is an escape. Many of these finishes have volatile organic compounds that may be toxic to the insects. VOC levels will be assessed during commissioning to minimize their toxic effects on the insects. Doors entering the insectary will be foam filled RFP with a viewing window and stainless steel door jambs and hardware. Armored plates will be on the door to minimize damage from equipment. All door assemblies will be sealed with 100% silicone. They will be 42" wide with a height of 7.5' with key card access to restrict entry to the Insectary suite. Door entries to the environmental chambers are automatic glass sliding doors. Opening of the automatic doors activates an air curtain that prevents insects from escaping the environmental chamber rooms. Doors close automatically. Sweeps will be installed to prevent vermin from entering the insectary suite. Lighting and electrical fixtures will be recessed and sealed with 100% silicone ASTM C920 to prevent vermin entry.

The lighting within the insectary and animal rooms (see Goal 2, below) will be broad spectrum UV lighting with dimmers to simulate dusk to dawn lighting. Photoperiod depends on colony requirements. This will be controlled through the building automation system (Johnson Controls). The light intensity of the environmental chambers will be 430-540 lux. When care is being provided, additional lighting to approximately 810 lux will be provided. This will be accessed through a timed override switch that will revert to 430-540 lux when time expires. Work rooms and other areas will be accessed via a local switch that provided suitable working light of 810-1075 lux. Lighting will be monitored by the BAS systems and accessible to the facility manager to make adjustments in the light cycle as needed. All electrical outlets will be flush to the wall, sealed and opening covered with screen mesh to prevent escapes.

Environmental controls will be provided by a dedicated HVAC system that has a minimum 20% allowance and an N+1 redundancy. This will permit air flow to be maintained independently of other areas of the CVID including laboratory space and administrative space. Air supply is 100% fresh air with HEPA filtration of supply air. Air changes are 6-10 air changes per hour in the insectary. Air flow of the environmental chambers will be maintained negative to the central corridor, and the insectary suite will be maintained negative to the rest of the building. A pressure independent constant volume control will be used to control the supply and exhaust flow rates to maintain the appropriately ACH. Exhaust air will have HEPA filtration to prevent insect escapes. All supply and exhaust will be low velocity design to minimize turbulence when handling insects. Airducts will be covered with a mesh screen to prevent insect escape the holding room, and minimize insect entry. Temperatures will be maintained between 72-80^{o}F with 25-55% relative humidity, depending on the arthropod species. Where higher levels of humidity are required, we will use portable humidifiers as described below. This will also be monitored by the BAS with alarm set points set at +/- 5 ^{O}F and +/- 5% RH. If environmental parameters exceed these limits, facility personnel will be notified of the alarm, and immediately assess the situation. Utilities to the insectary (located in the CVID penthouse) will be on emergency backup power.

An effluent decontamination system (EDS, or "kill tank") will be constructed to minimize the likelihood that immature mosquitoes and other arthropods will be released from the insectary into the environment via the plumbing. The proposed EDS is a centralized chemical treatment system. Chemical treatment EDS is less expensive than the more common thermal EDS that uses steam. The proposed system consists of (1) two

polypropylene treatment tanks with internal agitation to mix chemical, (2) chemical pumps, (3) control valves and (4) a Programmable Logic Controller (PLC). The system will include sodium hypochlorite injection for biological inactivation, and sodium thiosulphate to neutralize the chlorine concentration after treatment. Note that even after neutralization, the chlorine concentration will be on the order of 600ppm and may require dilution with water at a ratio of 10:1 to reduce chlorine levels down to acceptable sewer discharge limits. Replaceable chemical storage totes will be provided by the users and will need to be replaced every 2 to 5 days depending on the amount of effluent flowing through the system. The system has been designed to reflect the size of the facility and anticipated effluent volume. This system does not require laboratory users for maintenance, however facility management personnel needs to maintain the system. In addition, Biological Indicator (BI) validation will be performed annually. The system will be installed underground, in a partial basement or in an underground vault. The vault must be accessible for maintenance and ventilated to remove chemical fumes.



EFFLUENT DECONTAMINATION SYSTEM FLOW DIAGRAM

Additional components of the insectary are:

Ten 121 SF environmental chambers. These rooms are used to house arthropod colonies, mainly mosquitoes. They are key to successful operation of the insectary because they allow us to keep all of our colonies in close proximity (they are currently housed in three different buildings) Ten rooms are required because different mosquito species have different temperature, humidity and photoperiod requirements. We currently possess *Culex pipiens, Cx. quinquefasciatus, Cx. tarsalis, An. gambiae*, and several *Ae. aegypti* and *Ae. albopictus* colonies. In addition, we anticipate adding *Culicoides* and *Ixodes* colonies to our insectary to support newly funded projects. Heat for these rooms is provided by the building HVAC. Photoperiod will be controlled from a remote location within the building. No light switches will be available within the chambers to minimize the likelihood of accidental interference with programmed photoperiod control. Hot and cold water will be present at a sink in each chamber. A single T valve at each sink will be used to feed house DI water into a mixed bed filter and into a portable humidifier, which will be connected to a sensor to control humidity. (This is our standard practice within the CSU insectaries since we have learned over the years that HVAC systems are frequently incapable of maintaining humidity and fail often.) These and other rooms within the insectary will have 8 foot fully sealed ceilings (including light fixtures) that are light in color to facilitate the capture of loose arthropods.

One 212 SF environmental chamber. This room is the same size as those above, and shares identical requirements and MEP specifications and controls. It differs in that is has an anteroom with an additional workspace (hence 212 SF) and an additional level of containment. This area is designed to house and contain transgenic mosquitoes and others that require additional containment.

One large cage chamber (385 SF). This room will be used for population cage studies. This type of room has been used to test the efficacy of various interventions in reducing mosquito abundance or altering population

structure. The MEP specs and requirements for surfaces and ceilings, lighting and humidity, etc., are as above. One large sink with hot and cold water is placed within this room. Humidity and temperature are maintained as above. Six large insect cages will be constructed within this room and are indicated as dotted lines in **line drawing 5.**

One wind tunnel (212 SF). This wind tunnel will be custom built to WHO specifications and is used to evaluate whether insecticide formulations are effective for indoor use. This room is generally similar to the environmental chambers described above. It requires additional electrical power to run the wind tunnel, which consists of a fan at one end that draws air at a uniform flow rate measured by an anemometer and controlled by a rheostat. A plume of insecticide is generated using an atomizer and allowed to enter the moving air column. A cage containing mosquitoes is placed at the other end of the tunnel and mosquito mortality is monitored. A fume hood is required in this room for preparation of insecticide formulations.

Work Rooms (292, 291 and 125 SF). These rooms are generally used for storing and preparing supplies for maintaining arthropods (e.g. larval food, bloodmeals, small cages, salt solutions etc.), performing routine tasks for these colonies (e.g. separating pupae prior to emergence, cleaning mass rearing bins) and collecting specimens for arthropod studies. Benches in these rooms are to accommodate dissecting microscopes and other small equipment needed for arthropod studies. The smallest workroom (125 SF) has one sink with hot and cold water and space for up to five stand-alone incubators and/or refrigerator-freezers. The 291 SF work room contains a BSC for cell and virus work and several large benches for inoculating mosquito embryos and other fine manipulations needed for arthropod studies. Additionally, electrical and space considerations have been provided for three incubators and/or refrigerator-freezers. The 292 SF work room is constructed to provide additional workspace for preparing supplies needed to maintain mosquito colonies. This will be the main support area for the insectary facility.

Microscopy room (78 SF). This will house a fluorescence microscope for studies involving transgenic mosquitoes that bear reporter constructs and those infected by viruses that have been engineered to contain fluorescent reporters.

Manipulation room (92 SF). This room is to accommodate the need for separate, quiet space to work with mosquitoes undisturbed.

C.2 Goal 2. Bat facility.

The animal holding facility for the CVID is unique in the species that it will primarily house, bats. CSU has been successfully housing Jamaican fruit bats (*Artibeus jamaicensis*) for nearly 5 years. This facility will permit us to expand the colony, co-locate the bats adjacent to the laboratory space, and consequently vacate existing space to accommodate additional large animal studies. The new bat area (**line drawing 6**), will consist of three animal holding rooms, a procedure space, and a food prep area. In total the space is 1070 GSF. The CSU bat colony originated from a closed colony from a near-by institution. The bats were relocated to CSU following the successful recruitment of the investigator. The colony has been maintained in captivity for over 12 years. The bats are maintained by Laboratory Animal Resources (LAR) under the direction of Lon Kendall, DVM, PHD, DACLAM. LAR provides daily care and veterinary care for the colony, currently consisting of 180 bats. Since arriving at CSU there has been no reported health issues associated with the bats. LAR will continue to provide daily care of the bats in the new animal holding suite in the CVID. While the animal suite is principally designed to house the bats, there is versatility in the design to accommodate other species, primarily small animals.

The CVID bat suite is located in the corner of the facility away from the laboratory and administrative spaces. The facility will be secure and have limited access via key card access. Key card access will only be provided after IACUC approval of the animal activities and the personnel, and after facility training by the animal care staff. Only those with key card access will be permitted in the suite. Limited access also supports the biosecurity of the animal holding, as does the directional air flow described below. The CVID animal suite is part of a larger animal holding suites within the Infectious Disease Research Complex at CSU where there are eight animal holding suites adjacent to procedure and laboratory space. Many at an ABSL3 containment level. There is a centralized cage wash facility that supports the animal care operation at the IDRC, which will also

support the CVID animal suites. The cage wash facility has two bulk sterilizers, a rack washer and a tunnel washer. There is also a centralized dock to receive animals and supplies, as well as a centralized storage for equipment and feed. Soiled equipment and materials are transported from the animal suites on covered carts to the cage wash facility to minimize exposure to personnel. Material coming from the ABSL3 suites is appropriately decontaminated prior to transport. Animal care personnel offices are located within the IDRC.

There is 599 sf of animal holding space in three rooms, all accessed from a central procedure room of 287 sf; and a 124 sf anteroom and food prep area. The rooms are all similarly designed with a 9' ceiling height. The walls and ceilings will be composed of smooth, reinforced fiberglass panels to facilitate sanitation. Joints between panels will be sealed with architectural urethane sealant (or 100% silicone ASTM C920) to make them impervious to moisture. The floor will be slip resistant and coated with an anti-skid additive to the epoxy coated finish. The epoxy coat will have a 6" lap over the wall to create a transition that will prevent leakage and facilitate sanitation.

To facilitate cleaning for the bats, which create quite a mess, the floor will be sloped at a pitch of ¼ inch per foot to a trench drain, with a 4" outflow drain. This pitch will facilitate cleaning but will not be excessive as to prevent other caging types, such as rodent racks, from being used in the space. Cleanouts are accessible outside of the animal space. Hand washing sinks are located in each of the animal rooms, procedure room and food prep areas for personnel use and cleaning and sanitation.

Doors will be foam filled RFP with stainless steel door jambs and hardware. Door levers will be used to facilitate easy opening of doors, compared to a knob. A viewing window with red tint will be provided to allow personnel to view activity in the room prior to entering. The viewing window with have a magnetic closure to prevent disruption to the animal holding while working in the procedure space. Armored plates will be on the door to minimize damage from equipment. All door assemblies will be sealed with 100% silicone. They will be 42" wide with a height of 7.5'. Sweeps will be installed to prevent vermin from entering the animal suite. Lighting fixtures will be recessed and sealed with 100% silicone ASTM C920 to prevent vermin entry and facilitate sanitation.

The lighting within the animal holding rooms will be broad spectrum UV lighting on a 12:12 light dark cycle with dimmers to simulate dusk to dawn lighting. This will be controlled through the building automation system. The maximum light intensity will be 270 lux when no work is in progress. When animal care is being provided, additional lighting to approximately 810 lux will be provided. This will be accessed through a timed override switch that will revert to 270 lux when time expires. Procedure space and food prep areas will be access via a local switch that provided suitable working light of 810-1075 lux. Lighting will be monitored by the BAS systems and accessible to the facility manager to make adjustments in the light cycle as needed. All electrical outlets will be water tight and explosion proof and protected from the bats with latched covers.

Environmental controls for the animal suite will be provided by a dedicated HVAC system that has a minimum 20% allowance and an N+1 redundancy. This will permit air flow to be maintained independent of other areas of the CVID including laboratory space and administrative space. Air supply is 100% fresh air with 95% filter of supply air. Air changes are maintained according to the Guide for the Care and Use of Laboratory Animals at 10-15 air changes per hour. A pressure independent constant volume control will be used to control the supply and exhaust flow rates to maintain the appropriately ACH. All supply and exhaust ducts will be covered with a mesh screen to prevent bats from escaping the holding room. The screens will also minimize insect entry.

Directional airflow of the animal holding rooms will be positive to the adjacent procedure space. The air flow of the anteroom and food prep areas will be negative to the procedure space. This will create an air lock within the procedure space to prevent cross contamination and maintain the air within the animal suite. When bats are infected, the room air flow of the animal holding room will be made negative to maintain biosecurity and biocontainment. Animal holding room temperatures will be maintained higher than the Guide recommendations to accommodate the tropical bats. The temperature will be maintained a 75-77^{O}F and a relative humidity of 50%. The temperature and humidity will be monitored and recorded daily by the animal care staff. They will also be monitored by the BAS with alarm set points set at +/- 5 ^{O}F and +/- 5% RH. If environmental parameters exceed these limits, facility personnel will be notified of the alarm, and immediately

assess the situation. As a fail-safe mechanism to prevent animal holding rooms from overheating, the reheat coils will be set to fail in the closed position. Utilities to the animal suite will be on the CVID emergency backup power system. Access to the utilities located in the CVID penthouse will be minimally disruptive to the animals and occur outside of the animal suite.

The procedure space adjacent to the animal holding will minimize animal transport and exposure to personnel. They will be outfitted with a class II A2 BSC to facilitate animal manipulations and maintain biosecurity and biocontainment. All casework and tables will be epoxy coated steel and moveable to facilitate sanitation. Personal protective equipment will be stored in this location for personnel prior to entering the animal holding space. At minimum personnel will don facility lab coats and gloves when working with the animals. Animal care staff will additionally don facility scrubs and dedicated boots for cleaning. The food prep area will have a refrigerator and counter for preparing bat food. Sanitation supplies will also be stored in this location. Within the animal holding rooms for the bats, a portable mesh screening system with aluminum frames will be used to separate the bats from the door so personnel can easily enter the space without bat escapes. These will be inset approximately 4' and run the length of the room (**line drawing 6**). The bats in the breeding colony will have free flight access to the room. Roost will be installed on the ceiling made of a dark plexiglass material to minimize light and facilitate sanitation. Multiple areas throughout the room will be designed for the bats to hang. We have successfully used tarps made of a polyester material that hang from the ceilings and are easily removed and sanitated in the tunnel washer. Similarly we will provide faux stalactites as environmental enrichment. These will be made of 2" PVC tubing 2-3' long that can be fitted into a PVC end mounted to the ceiling. They can be easily removed for sanitation. All penetrations will be properly sealed with silicone. Bats that are used for studies, primarily infectious agents at an ABSL2 level, will be housed in smaller flight cages located with the room. They will be designed similarly to the mesh screen system described above with aluminum frames. They can be sanitized in place or deconstructed and taken to the cage wash facility. Caging for other small mammals will use our existing inventory of Tecniplast caging systems for mice and rats. This animal suite will provide a unique area to house and use bat animal models.

C.3 Goal 3. Other Key Enhancements to the CVID.

Autoclave and Glasswash spaces. This 423 SF support area (**line drawing 7**) is located between the insectary and tissue culture suites. This space and the autoclaves within it will support the entire CVID building that will house at least nine PIs and labs, including approximately 50 trainees and staff.

Three large autoclaves will be purchased and installed in this space as indicated in **line drawing 7**. Since this is a wet area, floors will be coated with epoxy resin to allow efficient washing. The floor will be slip resistant and coated with an anti-skid additive to the epoxy coated finish. The epoxy coat will have a 6" lap over the wall to create a transition that will prevent leakage and facilitate sanitation. Autoclaves will be provided with medium pressure steam for decontamination and floor drains for waste discharge. A central steam system also is used for building heating and domestic water heating. Autoclaves will be used for sanitizing glassware and solutions prior to use and decontaminating tissue culture and other biohazards prior to disposal. Three are requested because the two we currently use are frequently backlogged due to usage volume and/or equipment failure. New autoclaves are thus required.

Tissue culture suite. This 2,164 SF facility houses five 207 SF rooms to support studies undertaken in other areas of the CVID **(line drawing 8)**. The rooms and the biosafety cabinets within them are critical to the CVID because they will allow us to isolate insect and bat cells and conduct key support assays for the insectary and bat areas. They will be used for nearly all research conducted within the CVID, as nearly all projects have some need for cell cultures. Importantly, these suites also will serve as prep stations for cells that will be used in the adjacent BSL-3 virology suite via the elevator (below), which will ease the burden on our tissue culture facilities within the BSL-3 laboratory. Floors and walls of tissue culture suites are similar to other BSL2 areas in the CVID and will be essentially as described above.

Elevator. This 66 SF space houses the elevator (**line drawing 9**). This elevator will allow carts to be easily moved between the CVID and cage wash and BSL-3 laboratories.

Closing summary.

The AIDL at CSU houses several robust and highly productive NIH-funded research programs that are addressing some of the most difficult problems facing public health and medicine. The types of problems that are addressed are likely to constitute an increasing burden due to increasing human populations, increases in travel and trade, the rise of tropical megacities, land use changes and increases in arthropod resistance to inexpensive and nontoxic insecticides. CSU has made a significant commitment to the future of our research programs and is planning for the long-term success of the AIDL research agenda. The funds requested as part of this application will leverage this investment to provide a truly state-of-the-art facility that will enhance not only our ongoing research, but provide badly needed opportunities for us to form the types of collaborative and interagency partnerships that will be required to meet the challenges posed by arthropod-borne and bat-associated infectious diseases.