**General Insectary Standard Operating Protocols (SOPs)**

Version of November 2016

**Insectary Description**

The Insectary Suite in the Bloomberg School of Public Health is located on the fourth floor of the Hygiene building. This facility occupies approximately 3,000 sq. ft. of space that is divided into several different work areas and procedure rooms (Fig. 1). In total the facility houses nine walk-in incubators used for arthropod colony rearing and feeding, a tissue culture laboratory, and ten stand-alone incubators where humidity, temperature and light can be controlled for specific experiments. These incubators are distributed in 5 general work areas as diagrammed in Fig. 1. Area 5 is used strictly for rearing mosquitoes only. No infections of any type are performed there. Among the general equipment distributed throughout the facility, Spinsect insect traps are located in every room including the walk-in incubators and hallway to monitor loose insects. Two -20°C freezers are available for immobilizing and killing mosquitoes: one in the main hallway and a second in the enhanced-security Area 2.

The entire facility functions under BSL-2 / ABSL-2 (arthropod BSL-2) conditions. Within the insectary facility, Area 2 was designed to provide higher containment and is where all potentially infectious blood feeds are carried out. Live infected mosquitoes cannot be taken out of Area 2. A pass-through autoclave is located in this area and Area 2 may be upgraded to ABSL-3 in the future should the need arise, but at this time it functions at ABSL-2 with additional physical barrier control (2-3 more doors with hanging plastic barriers). All work currently conducted in the insectary is at the BSL-2 or lower level.

**Personnel and Access**

Access to the insectary facility is permitted to those individuals actively conducting or supervising work in the facility. Swipe card access to the general facility is possible at one of three points. An additional swipe card access is necessary for entry into Area 2, after proper training and certification have been completed. All personnel working within the facility must be familiar with the insectary use guidelines and restrictions and both the general SOPs and any specific SOPs required for their investigations.

All personnel must be thoroughly familiar with all relevant SOPs (this one and all laboratory-specific ones relevant to the person’s activities) and be properly trained by the Insectary Manager in relevant insectary procedures before work is initiated in the insectary. Swipe card access will be activated once the requester is properly trained. Access to the enhanced-security Area 2 is only granted to investigators with appropriate training certificate (see below).

**Insectary BSL-2 Guidelines [modified from the Biosafety in Microbiological and Biomedical Laboratories (BMBL)]**

**Biosafety Level 2** is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-1 in that (1) laboratory personnel have specific training in handling pathogenic agents; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment. The following standard and special practices, safety equipment, and facilities apply to all activities conducted in the insectary:

A. *Standard Microbiological Practices*

1. Access to the insectary facility is limited or restricted to those appropriately trained.

2. Personnel must wash their hands after they handle potentially harmful or infectious materials, after removing gloves, and before leaving the laboratory. Gloved hands are not used to open any doors.

3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas.

4. Mouth pipetting and aspiration of arthropods without appropriate protection is prohibited; mechanical pipetting devices must be used when appropriate protection is not feasible. Suitable mechanical / barrier aspiration devices include motorized, vacuum-assisted and HEPA-filtered aspirators.

5. Policies for the safe handling of sharps are instituted and need to be strictly followed.

6. All procedures are performed carefully to minimize the creation of splashes (e.g. larval rearing pans) or aerosols.

7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of potentially harmful or infectious materials or blood with disinfectants that are effective at eliminating any potential contamination.

8. All regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

B. *Special Practices*

1. Access to the laboratory is limited or restricted to authorized individuals when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the insectary, prep, culture or rearing rooms. For example, persons who are immunocompromised or immunosupressed may be at increased risk of acquiring infections. Only personnel proficient with the protocols and needed to carry out infection associated procedures should be in an area of potential exposure.

2. Only persons who have been advised of the potential hazards and meet specific entry requirements (e .g., immunization) may enter the laboratory.

3. A biohazard sign must be posted on the entrance to the laboratory where etiologic agents are used. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations or treatment, the investigator’s name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.

4. Insectary personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory.

5. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel may be collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.

6. The procedures specified in this document must be followed in addition to any investigator-specific protocols.

7. The principal investigators ensure that all personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive updates or additional training as necessary for procedural or policy changes. A form certifying appropriate training of each insectary worker must be kept on file.

8. High precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative. Plasticware should be substituted for glassware whenever possible.

b. Only needle-locking syringes or disposable syringe needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

c. Syringes which re-sheathe the needle, needle-less systems, and other safety devices are used when appropriate.

d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

e. All disposable glassware used for preparation of *P. falciparum* cultures should be disposed of in an appropriate sharps container within the biosafety cabinet.

9. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.

10. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant (eg. 70% ethanol, 10% bleach for malaria) on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

11. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the immediate supervisor and insectary director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

C. *Safety Equipment* (Primary Barriers)

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:

a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonated eggs.

b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.

3. Full-length trousers, sleeved shirts and close-toed shoes are required to be worn at all times (see below for details). Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.

4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching “clean” surfaces (keyboards, telephones, door handles, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

**Additional ABSL-2 Insectary Guidelines**

1. Compliance to all BSL-2 conditions as detailed in the preceding section.

2. To the best of our abilities, personnel working in the insectary must actively contribute to achieving zero loose mosquitoes in any part of the insectary. This means check any room you are in and ensure that there are no loose mosquitoes before you leave that room, whether yours or someone else’s. This applies to ALL ROOMS within the insectary facility. It is recommended that loose mosquitoes be caught using a 70% ethanol spray bottle to spray the mosquito as it alights on the wall or alternatively by swatting. See next paragraph for disposal instructions.

3. No mosquito carcasses should be left anywhere. All dead mosquitoes from experiments, dissections and old cages **MUST** be bagged or wrapped before being discarded into a biohazard trash box. **Make sure there are no loose mosquito carcasses anywhere in your work area before you leave it**. Disposal of adult or immature mosquitoes into the sink is not acceptable. If a mosquito is squashed with swatter against wall be sure to wipe the dead carcass off the wall.

4. **NO LIVE MOSQUITOES ARE TO LEAVE THE INSECTARY AT ANY TIME.** Arthropods can be freely moved within areas of the insectary facility reserved for un-infected specimens, using either metal or plastic cages or in cardboard “ice cream” cups as long as the tops are securely closed.

5. Turn off lights when you leave any area to help the Spinsects catch any loose mosquitoes. Spinsects will be monitored for loose arthropods.

6. Housekeeping is kept out of the insectary for many reasons including traffic control. This means we need to clean up after ourselves. For now you are responsible for the area in which you work. Make sure the trash is secured and disposed of as necessary. Dry Swiffers are available to clean floors.

7. As in any laboratory, the standard procedure is to wipe down and clean your area before you leave it. ALL countertops must be wiped down and cleaned as needed.

8. Police each other – in a positive way if possible. The insectary is used by a large number of personnel from different labs and backgrounds, but the public image of the insectary to the world around us is of a single unit. Therefore, even a few loose mosquitoes or a dirty work area in one room reflects poorly on all of us.

9. Remember that the insectary is a BSL2 facility – therefore all personnel must comply with the appropriate attire as detailed below. Consult with your supervisor if you need clarification of these and other personal safety issues.

10. **Labeling**. All arthropod containers, whether colony or experimental must be fully labeled with the species contained, appropriate dates, infection status (what pathogen and when infectious feed was done) and investigator’s name.

**Proper Attire for Laboratory Personnel**

**[Source: Johns Hopkins Safety Manual Policy HSE 801** <http://www.hopkinsmedicine.org/hse/Policies/HSE_Policies/indiv_sections/hse801.pdf>.**]**

It is the policy of Johns Hopkins that all employees, faculty, students and visitors wear appropriate attire in all laboratory areas to minimize or eliminate skin contact with hazardous materials.

Shorts, miniskirts or any apparel that does not cover the skin above the knee when seated shall NOT be worn in the laboratory or insectary facility without appropriate over protection. (eg. a buttoned laboratory coat or closed front gown.) Open toed shoes, sandals or shoes made of loosely woven material shall not be worn in the laboratory. Gloves shall be worn whenever there is a potential exposure of the hands to hazardous materials. The gloves must afford the necessary resistance to the hazardous material being used. Gloves must be removed before leaving the laboratory.

Specialized protective clothing shall be worn when using materials that are extremely hazardous upon contact with skin. Health, Safety and Environment (955-5918) should be consulted for these materials.

Additional regulations that may apply are the Use of Protective Eye and Face Equipment (HSE 007) and the Animal User Policy (HSE 034).

The use of complete cover of arms, legs and foot is highly recommended while in the insectary.

**Work with *Plasmodium falciparum***

1) Any person contemplating work with *P. falciparum*-infected mosquitoes must undergo formal training and receive a written certificate of training completion. At present, training will be administered by Dr. Godfree Mlambo. Card access to the enhanced-security area will only be provided after completion of training.

2) All work with *P. falciparum*-infected mosquitoes must be performed in the enhanced-security Area 2. No live infected mosquitoes are to leave this area.

3) Prior to infection, mosquitoes are to be distributed into the experimental containers (e.g., small metal cages, “ice cream” cups) in any insectary area OUTSIDE Area 2. This is to minimize the possibility of mosquito escape in the enhanced-security area.

4) IMPORTANT. It is essential that the exact number of mosquitoes be entered onto the container label PRIOR TO feeding with a *P. falciparum*-infected blood meal. This will allow for control of any losses that may occur. This number must be verified at the time of dissection or killing at the end of the experiment. Any discrepancy should be reported to the Insectary Director and/or Manager. This rule applies to any experiment in which 50 mosquitoes or less are handled per container.

5) All *P. falciparum*-infected mosquitoes MUST be kept double-caged (ie., a container within a container) at all times.

6) A container of *falciparum*-infected mosquitoes should never be opened, unless the mosquitoes have been PREVIOUSLY IMOBILIZED, for instance by placing them in the -20 oC freezer in the secure facility for 3~5 minutes. This should prevent unintended mosquito escape.

7) Everyone must remain vigilant and watch for loose mosquitoes in the enhanced-security Area 2. If one is found, it should be killed (e.g., by 70% ethanol spray) **AND DISSECTED** to determine whether it is infected or not. Regardless of infection status, the killing of a loose mosquito in the enhanced-security area should be recorded in a log that is provided. If the mosquito is found to be infected, the PI and the Insectary Director should be notified. A loose infected mosquito will trigger malaria prophylaxis (chloroquine) of everyone who has entered the enhanced-security area.

**Contact Information**

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