

Appendix 6

BUMC STANDARD OPERATING PROCEDURES

The following standard operating procedure is an example of the level of detail that will be required for every SOP for the proposed Boston NBL facility. This example is for a U.S. Centers for Disease Control Prevention (CDC)/U.S. Department of Agriculture Select Agent and Biosafety Level 3 agent currently being used at Boston University Medical Center (BUMC). For the purposes of this example, the name has been removed for security reasons, and will only be designated as "X bacteria." All Select Agent work will be approved by the BUMC Institutional Biosafety Committee, including the NBL Select Agent work.

Standard Operating Procedures For Experiments with Select Agent X

I. Project Description

The major objectives of this research will be to: (1) to develop a vaccine candidate to protect against inhalation of X (caused by X bacteria). (2) to develop a polyclonal antibody library to be used for passive immunization to ameliorate or prevent acute illness from X bacteria acquired by the inhalation route and (3) to develop diagnostic systems to detect X bacteria in clinical specimens and in the environment using immunochemical and/or gene amplification methods.

Project 1:

The overall research program is made-up of three sub-projects and two cores. In project 1 we will prepare and use as vaccine candidates in experimental systems, X bacteria lipopolysaccharide derived O-polysaccharide and capsular polysaccharides. We will use conjugates and clinically relevant adjuvant and delivery systems to recruit T cell help to enhance immune responses. We will also create peptide surrogates (called mimics) of the two saccharide prototypes and use these for immunization. Mice will be immunized to assess vaccine efficacy against aerosol challenge with X bacteria.

Project 2:

In project 2, we will design polyclonal antibody expression libraries (PCALs) against X bacteria and examine the efficacy of passive administration in preventing and treating experimentally induced inhalation tularemia. We will use widely directed polyclonal antibodies to determine overall efficacy, use libraries depleted of putative subversive (blocking) antibodies and generate monospecific polyclonals directed against O-polysaccharides and capsular epitopes to passively immunize mice and assess protection from aerosol challenge with X bacteria.

Project 3:

In project 3, we will develop three diagnostic systems, using gene amplification and immunochemical detection, to detect X bacteria in clinical and environmental specimens. A transcription mediated amplification (TMA) assay will be developed to detect X bacteria in respiratory secretions and an immunochemical test to detect X bacteria antigens in respiratory secretions and urine and for monitoring environmental air samples.

Core A:

The Core A (Animal Core) will support research on X bacteria in mice to develop a vaccine candidates (developed in Project 1). Vaccine candidates that yield a high immune response will be used to vaccinate mice prior to aerosol challenge with living organisms. Vaccine efficacy against aerosol challenge will be assessed on the basis of survival and quantitative organ cultures. In addition, Prophylaxis and therapeutic efficacy of the PCALs (developed in Project 2) to aerosol challenge with live organisms will be performed on mice. Urine and respiratory secretions from infected mice will be used to optimize the development of diagnostic assays (Project 3)

Core B:

The Core B (Bacteriology/Immunology) will provide the centralized bacteriology support needed for each of the projects and Core A.

II. LABORATORY FACILITY**A. Biocontainment Suite**

The Biocontainment Suite is a BSL-3 lab facility, consisting of 6 lab rooms, shared by one common hallway, a main room containing storage space, common sink and double-door autoclave, airlock, and common locker change room. This Suite is located on the xth floor of the XYZ building which requires card access from the 1st floor level elevator as well as the elevator foyer on the xth floor.

The Suite will be separated from areas that are open to unrestricted traffic flow within the XYZ building. Entry will be restricted by card key access or biometric scanner. The Suite will operate under negative-pressure at all times (see emergency response plans for failure or loss of power).

Due to the isolated nature of this 6- lab multi-room space, OEHS recommends that researchers do not work alone in the space for any aerosol challenge work or large manipulations of Tularemia culture. Should a researcher need to work alone, OEHS will require that personnel notify a person when they enter and exit the space. That person they are notifying must be on-campus during the time that the work is being completed. Should a worker have a medical injury or fail to report back, the colleague should immediately contact the Control Center (4-6666).

B. Work Space for X bacteria Experiments

Work space will consist of 3 rooms (A, B, & C) within the biocontainment suite (A-bacteriology laboratory, B- procedure room, and C-Animal housing).

1. Common Space

There is a common hallway leading to these three labs. There is a shared autoclave in the common entry/supply room.

2. Safety Equipment

There is a safety shower, newly placed in the common hallway that shares all 6 labs. There is an emergency phone and flip chart located in the hallway. There is an eyewash, hands free sink, and telephone in each individual lab room.

III. ROLES AND RESPONSIBILITIES

A. Program Director-

The Program Director will be responsible for the following.

- Identify a Laboratory Director to provide supervision
- Assign a Laboratory Supervisor(s) to oversee daily operation of the laboratory
- Maintaining communication with LASC, OEHS, Facilities Management, IACUC & IBC committees.
- Communicating with LASC as necessary to review issues of animal welfare, operations, and compliance.
- Ongoing communication with other PIs to ensure that all users of the BSL-3 suite are familiar with the SOP, training, and other requirements of the laboratory.

B. Supervisor

The Laboratory Supervisor, (for Core A) will be responsible for:

- operating the animal housing room and the procedure room.
- He will ensure that all animals housed in the laboratory are properly cared for according to the standards of the Laboratory Animal Science Center (LASC).
- He will perform or train staff thoroughly on all aerosol infection studies, all tissue harvesting procedures, and all daily animal care.
- He will be responsible for tracking all animals used by the laboratory, for decontamination of all waste generated by the laboratory.
- He is also responsible for maintaining all select agent inventory records, and notifying the RO of any discrepancies.
- The Laboratory Supervisor (for Core B) will be responsible for operating the bacteriology laboratory. He will supervise the personnel working in the laboratory to maintain the lab according to the standard operating procedures. He will be responsible for keeping the lab according to the standard operating procedures and for keeping the laboratory supplied.

C. LASC Veterinary Manager

Provide oversight to all aspects of animal health and welfare and review and approve SOPs in accordance with the IBC and IACUC committees.

D. Security

The security department will be responsible for completing a risk assessment of the space, prior to the laboratory opening, and as needed. Security will also be responsible for monitoring the activity of the exterior locations of the storage space and lab, granting card access authorized by OEHS, and issuing color coded Select Agent ID cards to the users. Security will contain the

outside of the laboratory in the event of an emergency, and will investigate any loss of select agent material in collaboration with OEHS, LASC, the Lab Director, and other parties as needed.

E. Office of Environmental Health and Safety

OEHS is responsible for the training of all staff on lab safety training, BSL-3 & select agent training. OEHS is also responsible for monitoring the Select Agent Program.

F. Responsible Facility Official (RO)

Director of OEHS, is the RO for BUMC. He is responsible for:

Inspecting all select agent records, and auditing these records at least twice per year.

He is also responsible for notifying the CDC of any changes to the X bacteria project.

G. Approved Personnel

Personnel will need to be approved by filling out an FD-961 form for FBI background clearance checks.

IV. Access to BSL3 Suite

Prior to gaining access, all personnel entering suite must receive required safety training for BSL3 hazards, fit testing for N95 or EHS approved respiratory protection, and all trainings listed in Section VI B. Access to rooms will be further restricted to the following persons in Sections A through E:

A. Members

Members of the lab who have received safety training specifically for the laboratory under the Laboratory Director's supervision.

B. Visitors

Visitors (facilities maintenance workers, inspectors) must be trained, fit tested, and then accompanied by an approved Select Agent lab member, only after approval of the Safety Office and Laboratory Director. Visitors must meet BUMC Security Clearance requirements (see attached Appendix A) and must be escorted at all times.

C. LASC Personnel

LASC personnel who have received safety training specifically for the tularemia project under the Laboratory Director's supervision will enter to monitor the condition of mice.

D. Maintenance personnel

Maintenance personnel will enter the laboratory only after communicating with the Laboratory Director or the Laboratory Supervisor. Prior to entry by maintenance personnel, the Laboratory Supervisor will ensure that all staff have been properly fit tested and trained, infectious materials are safely stored, (i.e. no work is out in the Biosafety Cabinet, all animals are in appropriate cages), no experimental procedures are in progress, and that all work surfaces are

sanitized. Maintenance workers must be escorted by an approved staff members at all times and will have the appropriate PPE on approved by OEHS at all times.

E. Other

No persons under age 18 are permitted in the laboratory.

V. Violations

Violations of safety and security rules will be reported to the Research Laboratory Director, LASC Veterinary Manager, and to the Office of Environmental Health and Safety (OEHS) Biosafety Officer and Responsible Facility Official, and Security. Serious repeated infractions will be grounds for denying further access to the laboratory. The Select Agent Response Policy SP-XXX will be used by BUMC Security in the event of a security violation.

VI. PERSONNEL TRAINING

A. Administration Responsibilities

The Laboratory Director is responsible for ensuring that all personnel working in the laboratory receive specific training in safe procedures for experimentation with X bacteria in the BSL-3 laboratory. The Laboratory Director will maintain a record of training session attendees. The Laboratory Director is also responsible for ensuring that all personnel have received whatever additional training may be mandated by OEHS, LASC, OEM, or IBC.

B. Training/ Other Requirements

- Lab Safety Training
- LASC Animal Training
- BSL-3 training- (Lecture and hands-on walkthrough)
- Select Agent Training
- OSHA Medical Clearance for Respirator Use by OEM
- Fit Testing through OEHS
- Occupational and Environmental Medicine- (medical evaluation & additional requirements)
- Biologicals Shipping Training (specifically required for all personnel that are responsible for shipping infectious materials in this lab.)
- Basic First Aid/ CPR Training

VII. Lab Operating Procedures

A. Entry Procedures

1. **Prior to entering:** workers must be sure the HVAC alarm is not sounding indicating exhaust failure.

2. **Entrance:** Rooms will be entered via the front corridor with card key access and/or biometric iris scanner.

3. Logbook: Personnel must indicate Name, Date, Time of entry, and the Work Room destination, and purpose of work.

4. PPE: All researchers will wear the following personal protective equipment:

- disposable Tyvec gowns
- double gloves
- shoe covers
- eye protection
- respiratory protection (For all entry and all work to the BSL-3 suite an N-95 or NIOSH approved respirator will be required).

5. Checks: Upon entering the Biocontainment Suite, the following items should be checked:

- Magnehelic gauges
- Telephone.
- Door Sweeps
- Supplies and reagents are available, open, and ready for use.
- Aerosol Challenge Sign-Must be posted on Door to Lab B prior to challenge.

6. Before beginning any work: Surface disinfect work areas with 70% Ethanol. Do not assume that any surface is clean. If any problem is noted, contact the previous person that has signed into the suite for assistance and notify the Laboratory Supervisor.

B. Exit Procedures

1. **Wipe down:** the work area with 70% ethanol.

2. **Collect:** biohazard waste bags for disposal. Bags are then sprayed down on the outside with 70% ethanol or 70% isopropanol disinfectant.

3. **Discard:** outer gloves in the biowaste bag, close the bag in laboratory room (A, B or C). Carry biohazard waste bags to the autoclave.

4. **Trash:** bags in autoclave in secondary containment, start cycle, then proceed to locker room exit door.

5. **Removal:** If any trays or materials need to be removed, they must be surface decontaminated by soaking with 70% ethanol, and placing in the airlock for pick up on the other side.

6. **Exit:** You must swipe your authorized BUMC ID upon exiting BSL lab hallway to activate motion sensors per security protocol. Exit through the ante room/ Locker room. Discard Personal Protective equipment in the following order: Tyvec and head cover. Next the respirator should be removed and discarded. Next, the employee should step over the floor area indicating going from "dirty" to "clean" area of the locker room, while one shoe cover

should be removed at a time upon stepping over the line on the floor. Lastly, the inner glove should be discarded after all other PPE is removed.

7. **Hygiene:** Employees must wash hands upon every exit, then sign out of logbook indicating departure.

8. **Airlock:** Materials can now be safely retrieved out of autoclave on clean side, and the airlock on the clean side of the lab.

9. **Autoclaved waste:** bags are placed in Biohazard cardboard boxes in the waste storage room on the Xth floor. These boxes are then picked up by Stericycle for transport to an off-site incinerator.

C. General BSL-3 Microbiological Practices

1. **Handwashing:** Hand washing is required after handling infectious materials and before leaving the suite.

2. **Double Gloves:** Use of double gloves is required. The outer gloves should always be changed immediately after handling potentially infectious materials and after any spill or accident.

3. **Restrictions:** Eating, drinking, applying cosmetics, inserting contact lenses, storing food, shorts and perforated shoes or cloth sneakers are prohibited in the lab.

4. **Decontamination Practices:** Work surfaces must be decontaminated after every use, and immediately after any spill of infectious material. Disinfectant solutions are kept in every work area. Bleach solutions should always be dated.

5. **BSC Use:** All procedures with infectious materials are performed in a biosafety cabinet or other physical containment device to minimize exposure to aerosols.

6. **Waste:** All infectious waste must be autoclaved before removal from the BSL-3 lab.

7. **Autoclave Validation:** Laboratory personnel will check autoclave performance monthly with a biological indicator. Biological indicators consist of ampoules containing heat resistant spores (*Bacillus stearothermophilus*). All usage of the autoclave needs to be recorded in a dedicated autoclave notebook located next to the BSL3 autoclave. The Ampoules of *B. Stearothermophilus* need to be placed inside the autoclave bag, once per month. Upon completion of cycle, vial is removed and placed in a 50°C incubator for 48 hours to verify there is no growth of organism. This validates the autoclave is sterilizing the waste appropriately.

8. **Contaminated equipment:** Equipment must be surface decontaminated by soaking with 70% ethanol in the airlock. On the occasional occurrence of trays or other equipment that needs to leave the BSL3 lab, the material must be brought to the air-lock, thoroughly drench-sprayed down with 70% ethanol spray, and surface wiped down. Upon exiting the facility, personnel may retrieve the item from the air-lock on the outer-containment door.

D. Aerosol Infection Experiments

1. **Intox:** The In-Tox Nose-Only Inhalation Exposure System will be used for inhalation exposure of mice to X bacteria. Only the Laboratory Supervisor, or persons trained and approved by the Laboratory Supervisor and the Laboratory Director, may operate the unit.

2. **Operating Manual:** A copy of the operating manual will be kept in the BSL-3 laboratory at all times.

3. **Signage:** A warning sign stating “Caution- Aerosol Challenge in Progress- X bacteria present” should be placed on the door of the lab when a challenge with the InTox system is taking place. This will prevent other persons from inadvertently entering the lab, and all lab staff and animal care personnel must be trained to recognize this sign. Note: it is important that lab staff are wearing the approved N95 respirator before entering the lab to complete the aerosol experiment.

There will be an additional sign placed on the locker room door and the airlock to indicate the PPE required to enter the space, even if emergency responder.

4. **Rodent Control:** Each mouse is put into an individual exposure chamber, face first, exposing the nose of the mouse out of the opening.

5. **Mice Removal:** Upon completion of the aerosol exposure to the mice, mice should be removed and placed back into cages.

6. **Disinfection of Chamber:** The interior of the chamber should now be cleaned with 70% ethanol solution.

7. **Disinfection of Surfaces:** Next all surfaces, floors, and countertops should be wiped down with 70% ethanol.

8. **Exiting:** Before leaving the laboratory, remove the above “Aerosol Exposure...” sign on the outer door to indicate experiment is complete.

E. Handling Infected Mice.

1. **BSC:** All work with infected mice will be conducted in the Class II, All or Class II BII biological safety cabinet that is hard-ducted and HEPA filtered to the HVAC system.

2. **Necropsy:** Necropsy procedures will be conducted with a minimal use of sharps. Scissors will be used in place of scalpels wherever possible. Blunt dissection will be performed in place of cutting wherever possible. Use of needles will be minimized, and needles will be never re-capped. An approved sharps disposal container will be present in the work area.

3. **Tissue Homogenization:** Homogenization of tissues will be conducted only in the class II biological safety cabinet.

4. **Tissue Safety:** After tissue grinding operations, wait 10 minutes before removing homogenate samples from the safety cabinet. Tubes should be covered and decontaminated on their exterior surface by spraying with 70% ethanol.

5. **BSC Decontamination:** After completing work in the safety cabinet, decontaminate the inside and outside of the BSC and chair with 70% ethanol.

F. Animal Care

1. **Laboratory Supervisor:** Supervisor, along with other designated and trained members of the laboratory works in collaboration with LASC personnel to ensure proper animal care. All routine animal care will be conducted with ABSL-3 practices.

2. **Rodent care:** Mice will be housed in micro-isolator cages, a ventilated HEPA filtered cage rack system purchased from Biozone, will hold 81 cages. There will be no more than 4 animals per cage. All mice will be visually inspected once daily for general appearance and for consumption of food and water. LASC personnel will conduct routine daily checks according to their requirements for general healthy condition of the mice along with room temperature and humidity checks. Any problems with the animals will be reported to the Laboratory Supervisor.

3. **Special requirements:** Room will be decontaminated and open for LASC inspection when necessary, in collaboration with researchers.

4. **Loose Rodents:** Any mouse found on the floor of the laboratory will be euthanized.

5. **Animal Care:** Lab personnel will change cages once every two weeks or as needed. Used cages, along with their bedding and water emptied water bottles, (while working inside a BSC) cages will be placed directly in biohazard bags for autoclaving. These bagged cages will be removed from the lab, then the outside of the bag is sprayed with 70% ethanol, and transferred to the autoclave for sterilization. Ultra high temperature cages and bottles will be used.

6. **LASC personnel:** LASC will retrieve autoclaved cages from the outer autoclave door outside containment. Bedding will be discarded and cages will be placed in the cage washer for future use. LASC personnel will supply fresh cages, bottles, and food to the Air Lock on a cart. The material will be left in the airlock, carts will be sprayed down with Quatricide disinfectant prior

to removal from the airlock by LASC personnel. An easily decontaminated plastic or stainless steel table may be dedicated to the airlock, and left in the airlock only to assist LASC personnel in providing clean animal cages and supplies for the lab staff.

7. **Lab Personnel:** lab will have dedicated stainless steel carts for the Suite that will never leave the suite. These carts can be surface decontaminated by soaking thoroughly all 4 sides with 70% ethanol, and also soaking the wheels. After surface decontamination is complete, sterilized carts can be wheeled into the airlock, in order to safely pick up clean cages and animal supplies left by LASC personnel.

G. Waste disposal

1. **Liquid waste:** Waste will be collected into tall plastic bins with lids, these bins will contain concentrated bleach solution to a final concentration of 10%. This decontaminated liquid waste bleach solution should be autoclaved prior to sink disposal with plenty of running water.

2. **Solid waste:** Waste will be collected in double biohazard bags and autoclaved. Autoclave standard cycle is 250°F (121°C) 15psi, for 30 minute cycle.

3. **Autoclave Validation:** Laboratory personnel will check autoclave performance monthly with a biological indicator. Biological indicators consist of ampoules containing heat resistant spores (*Bacillus stearothermophilus*).

4. **Autoclave Logbook:** Every use of the autoclave by Biocontainment Suite laboratory personnel must be recorded in a logbook, indicating the date, type of waste, operator, and result of biological indicator test, if used. Monthly biological indicator tests must also be recorded in the log. OEHS will train staff on proper autoclave use, including logbook entry and validation procedures. OEHS will monitor the autoclave validation throughout the year. After autoclaving sharps boxes, they are placed in the medical waste room on the Xth floor for BUMC Facilities pickup.

5. **Animal Carcasses and Sharps:** Carcasses will be placed in double biohazard bags and autoclaved. Sharps will be collected in an approved Sharps waste container which is puncture-resistant, and the container is autoclaved. All waste is placed in the medical waste room on the Xth floor for incineration pickup by BUMC facilities.

H. Removal of materials from the facility

1. **Research materials:** Research material removed from the facility must be decontaminated by autoclaving or by chemical disinfection. Autoclaving is the preferred mode and should be used for all refuse and labware. Items that cannot be autoclaved must be decontaminated by spraying the surfaces thoroughly with 70% ethanol or 10% bleach.

2. **Secondary containment:** Any samples transported into the lab will be transported only in sealed plastic containers, placed in a durable leak-proof container with a lid and may require a security officer validate the transporter as wearing an authorized select agent color coded ID and may require an escort to a different location if leaving the storage containment area to another building location.

3. **Container Removal:** Any removal of containers from the BSL-3 lab must be surface decontaminated. No live samples may leave the BSL-3 laboratory without prior authorization from OEHS. This includes samples that are transported from the basement storage area to XXX room. Any samples transported from XXX must have two approved personnel completing the transfer, and logbook must indicate this as required. The exterior of the primary containers (tubes) will be sprayed down with 70% ethanol or an OEHS-approved disinfectant prior to removal from the biosafety cabinet. These samples are placed in the secondary container. The container is brought to the airlock where the entire outside and inside is sprayed down with 70% ethanol before removal from the facility.

4. **Equipment Decontamination:** Lab Equipment will be decontaminated by autoclave or surface decontamination before removal from the facility for repair, maintenance, or packaging for transport.

VIII. Emergency Procedures

A. Loose Animal Procedure

In the event that an animal escapes from a micro-isolator cage, the animal will be trapped in the room by the door sweeps and will be caught and euthanized by the Lab personnel. Should the lab personnel need assistance, they should contact LASC for a veterinary manager and notify the Control Center so security can contain the area to prevent further injury. There will be detailed trainings as to how this functions with Security, Control, and the researchers involved.

B. HVAC Failure

In the event of a failure of the HVAC system, the HVAC system will sound an audible alarm indicating failure. Lab personnel are asked to immediately cease all lab work, secure any animals or select agents, and proceed calmly to the locker room, following normal exit procedures. Upon exiting personnel should call the Control Center (4-6666) to report the HVAC failure. There will be detailed trainings as to how this alarm functions with Security, Control, and the researchers involved.

C. Biosafety Cabinet Failure (BSC)

In the event of a failure of the BSC, the BSC alarm will sound an audible alarm. Lab personnel are instructed to immediately cease all lab work in the Biosafety cabinet, and secure any animals or select agents. There will be detailed trainings as to how this alarm functions with Security, Control, and the researchers involved.

Also, lab personnel may note that when the BSC alarm begins, air will be flowing towards them when sitting in front of the cabinet. This is an excellent physical indicator of an alarm failure. After securing materials, personnel should proceed calmly to the common autoclave room, call the Control Center (4-6666) to alert them of the BSC failure, then proceed to the locker room, following normal exit procedures. There will be detailed trainings as to how this alarm functions with Security, Control, and the researchers involved.

D. Aerosol Chamber Failure

In the event of a failure of the Aerosol Chamber, Lab personnel are asked to immediately cease all lab work. Personnel should secure any animals or select agents, and step into the common corridor. Personnel will be asked to push the yellow "Emergency Exhaust" button next to the emergency exit. This button will activate the exhaust fan to increase their flow rate from 60% to 80% from the lab. Personnel should pick up the emergency phone in the hallway, and call the Control center at (4-6666) to notify them of this failure. Then, if personnel are contaminated, they should identify to Control they need an emergency responder. The Control Center technician will alert Security staff that an SA incident has occurred, and security staff will follow Select Agent Response Plan as trained and will contact EMS and BFD to assist.

Lab personnel should drop all contaminated PPE (Tyvec suit and outer gloves) in the common hallway next to Lab B door. Personnel should proceed to autoclave room 935, pull decon shower from wall, and with hose in sink, proceed to soak themselves with water and soap provided. Upon completion of this, personnel should step into airlock where emergency responders will be waiting. Emergency responders will assess the situation, and if the person is contaminated, they will be suited in clean Tyvec and brought over to the Menino pavilion for medical treatment. There will be detailed trainings as to how this alarm functions with Security, Control, and the researchers involved.

E. Facility/ Building Failure

For any building related failure, including outside fire alarms, lighting, elevators, and similar, Personnel should call the Control (4-6666) to be alerted to the specifics to determine if they affect the BSL-3 space prior to entering/exiting. If personnel are already in the space and note a building problem, they should call the Control (4-6666) to determine if the problem will or could affect the space. In the case of a fire alarm outside the lab, personnel should follow normal exit procedures and immediately exit the lab and take the nearest stairwell exit.

F. Security Breach

If lab personnel note any discrepancy in the logbook or select agent storage area, they should immediately contact Security at 4-4444. Security will follow the Select Agent Response Plan, and notifying their supervisor immediately. A Security supervisor will be required to contact the OEHS approved staff to report the potential breach. Also if there is an intruder into the space, lab personnel are advised to stay in their laboratory and call Security immediately at 4-4444. There will be detailed trainings as to how this functions with Security, Control, and the

researchers involved. Security monitors will receive a motion detection alarm which will alert security to CCTV monitors and review last card or iris scanner used.

G. Fire in the Suite

An audible alarm will sound in the common hallway and strobe lights will initiate in each individual lab to indicate fire alarm. Lab personnel are asked to immediately cease all lab work and proceed calmly to the locker room. Personnel can then quickly remove all PPE in the airlock and exit the laboratory. In the event that exit way is blocked or the fire danger is great, personnel should exit the nearest exit, through the airlock or the emergency exit. However, personnel should drop all PPE at the exit door or inside the airlock. Upon exiting personnel should proceed down nearest exit stairwell and exit the building. There will be detailed trainings as to how this alarm functions with Security, Control, and the researchers involved.

H. Spills and Accidents

There will be detailed trainings and mock scenarios as to how this functions with Security, Control, and the researchers involved.

1. Disinfectants:

Before beginning work, check to see that a disinfectant solution is on hand and that it has not expired. If there is insufficient quantity then prepare 70% ethanol (preferred) or a 1:10 dilution of bleach containing 5.25% sodium hypochlorite.

2. Spills inside a biological safety cabinet:

- a. Step out of lab, Put on a clean protective gown (if necessary) and clean outer gloves.
- b. Spray or wipe walls, work surfaces, and equipment with a disinfectant solution.
- c. Flood the top work surface tray and the drain pans and catch basins below the work surface with a disinfectant solution and let it stand for 20 minutes.
- d. Remove excess disinfectant from the tray by wiping with a sponge or cloth soaked in disinfectant. Drain the tray and wipe the top and underside surfaces with a sponge or cloth soaked in disinfectant. Drain disinfectant from the cabinet base into a leak-proof container and let it stand for an additional 20 minutes before discarding in the sink.

3. Spills outside a biological safety cabinet:

- a. Step out of lab room, covering spill with paper towels if able to upon exit.
- b. Warn all others present in the BSL-3 not to enter the contaminated area by posting a sign on the lab door.
- c. Wait 30 minutes to allow dissipation of aerosols created by the spill. While waiting, call the Lab Director and the OEHS. Retrieve the spill kit and other spill clean-up materials from the common autoclave room.

- d. Put on a clean protective gown and double gloves before re-entering lab. Place Paper towels over spill, and soak with disinfectant. To minimize aerosol formation, avoid pouring disinfectant directly on the spill. Let stand 20 minutes to allow adequate contact time.
- e. Using an autoclave-resistant dust pan and squeegee transfer all contaminated material, including the dust pan and squeegee into a deep autoclave pan and autoclave promptly according to the standard directions.
- f. Spray down floor again with disinfectant, and wipe down all surfaces and equipment with disinfectant.

4. Large Spill Response:

- a. Step out of lab, immediately press "Emergency Exhaust" button located next to Emergency Exit Door. Drop all contaminated Tyvec and other PPE in hallway outside lab door.
- b. Pick up emergency phone call Control (4-6666) to report large spill. Control will follow Security Plan for Select Agents, and will immediately contact the OEHS- approved Safety personnel listed on-call for this area, to respond to the spill clean-up.
- c. If personally contaminated, personnel should alert Control during the phone call, they will alert emergency responders to the area as part of the response to the spill. Proceed with Decon procedures as described in Section D-Aerosol Chamber Failure.
- d. Exit lab according to same procedures in Section D-Aerosol Chamber Failure.

5. Medical Emergency:

If a worker becomes unconscious in a laboratory, a co-worker must call Control (4-6666) to alert them medical attention is needed. Personnel are asked to drag worker to airlock, or in the case of greater difficulty, drag to the emergency exit door, and cut with scissors the Tyvec gown from the worker in the common hallway. Emergency responders can meet the personnel in the airlock or at the emergency exit doorway to seek medical treatment. The common hallway floors and walls can be decontaminated as necessary after the incident.

*If an unconscious worker will be too difficult for the person to move, Personnel should call Control (4-6666), and wait with the downed worker. Trained emergency responders will be escorted by Security to the lab entrance, and will be able to enter the space with appropriate PPE to remove the unconscious worker. In this case, the space will be decontaminated as necessary after the incident.

6. SOP Manual and Emergency Instructions

A copy of the SOP manual will be kept in the BSL-3 laboratory at all times.

Instructions for responding to spills and emergencies will be posted in the BSL-3 laboratory. Emergency telephone numbers will be posted in the laboratory and in the common hallway next to the emergency phone.

IX. Maintenance, Cleaning, and Inspection

A. Maintenance

1. **Laboratory Director:** Responsible for ensuring that the class II BII Biosafety cabinets are certified on a yearly basis.

2. **Facilities Management:** Responsible for inspection and maintenance of the ventilation system, including monitoring the condition of HEPA filters on the roof of the building, and checking the eyewash, safety shower, and fire extinguisher annually which are all outside the containment area in 990 hallway. If access to area is required, it will only be with prior approval by OEHS and escorted by OEHS or their designee.

3. **LASC:** Assist laboratory personnel in maintaining rodent health.

4. **OEHS:** Responsible for safety of BSL-3 and training all staff. Safety will require that the Suite be decontaminated on an annual basis for general maintenance. This will include: Certification of the BSCs, Aerosol Chamber, Autoclave, Fire alarm, HVAC alarm, ventilated cage rack system, visual and mechanical checks of the plumbing, walls, and ceilings in the suite.

B. Cleaning/ Disinfection

1. **Laboratory personnel:** Responsible for daily housekeeping activities, including trash removal in rooms. LASC staff will not enter these areas for routine cleaning.

2. **Decontamination:** Work surfaces are decontaminated when work is finished, at the end of every workday, and immediately after any spill of infectious material. Large equipment will have inner and outer surfaces wiped with disinfectant weekly.

3. **Work surfaces:** The biosafety cabinet, glovebox, and aerosol chamber are decontaminated when work with infectious materials is finished.

4. **Sinks:** Sinks are scrubbed down weekly with a disinfectant and then flushed.

5. **Floors:** The floors are wiped down with 10% Bleach solution once each month, and immediately following any aerosol infection experiment. Wet mopping is the only approved method; dry mopping and sweeping are prohibited.

C. Inspections

The Laboratory Supervisor will conduct a daily inspection for general cleanliness, (See attached checklist) and to confirm that all interlocking doors close properly, that all airflow indicators are in the desired range, that the class II biological safety cabinets are operational, and that all mice are contained within their cages.

Any minor deficiencies will be corrected and any major deficiencies will be reported to the Laboratory Director, LASC, OEHS, and/or Facilities Management if needed. The Laboratory Director will conduct a monthly inspection with the Laboratory Supervisor and review the SOP. The Biocontainment Suite laboratories will be decontaminated and opened for inspection by personnel from LASC, OEHS, Facilities Management, IACUC, or the IBC at any time this is requested, within a reasonable amount of time for all parties involved.

List of Appendices (Not included in this document)

- Appendix A: Security Plan for Select Agents
- Appendix B: Biosafety Plan for Select Agents
- Appendix C: Emergency Response Plan for Select Agents
- Appendix D: Floor Plan XXX
- Appendix E: Medical Surveillance

Note: Appendices A-C are required for the Select Agent Program, and are kept secured in the Office of Environmental Health and Safety due to Security concerns. Appendix E is a Medical Surveillance protocol developed by Occupational and Environmental Medicine for responding to researcher exposure to X bacteria. The security plan should remain with the OEHS manual as it is secure information regarding security for the space.