Center for Infectious Disease Research

TB BSL-3 Facility Safety Manual

Version 1/2017

Version Number	Effective Date	Revision items / Comments
v.1/2017	**1/27/17**	Updated to reflect branding for AccelTB/PREEmpt; Change Seattle BioMed to CID Research; Add Revision Table and assigned version numbers*
v.6/2016	6/1/16	Modification to heat killing procedure; Revised administratively by EHS, Biosafety Officer & TB BSL-3 Director from IBC meeting discussion
v.11/2014	11/24/14	Removal of TBQ disinfectant from the Manual and full change to use of LopHene; Revised administratively by EHS, Biosafety Officer & TB BSL-3 Director

* Version number = *v. month/year* the Manual is revised.
Example: The manual is revised in December 2016 the version number is “v.12/2016”.

TB BSL-3 Facility Safety Manual

People.

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TB BSL-3 FACILITY SAFETY MANUAL

BIOSAFETY LEVEL 3 (BSL-3) FOR TB RESEARCH

I. Introduction & Research Program Requirements

This manual outlines the requirements for performing research involving the use of infectious *Mycobacterium tuberculosis* (TB) or *Mycobacterium bovis* in Center for Infectious Disease Research's TB BSL-3 Laboratory Facility. These procedures are designed to accompany the Biosafety section in the Center for Infectious Disease Research Safety Manual. The manual follows specific guidelines for the handling of agents described in the Centers for Disease Control (CDC) Publication: *Biosafety in Microbiological and Biomedical Laboratories (5th Edition)*, and complies with the *NIH Publication: NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*. In addition, procedures and the facility have been designed to ensure compliance with the Bloodborne Pathogen Standard WAC 296-823.

Only personnel who are competent scientists and are trained in handling with these agents will be certified to work with these agents in the BSL-3 laboratory. All procedures that involve manipulating infectious material are conducted within biological safety cabinets or other primary containment devices by personnel wearing appropriate protective clothing and devices. BSL-3 laboratories have special engineering and design features, as required by the CDC and NIH. Standard and special safety practices and equipment are described in the following pages and a map of the Center's BSL-3 laboratory is also appended.

A. Roles and Responsibilities

BSL-3 Director - The BSL-3 Laboratory Director is responsible for oversight of the facility and research operations. The Director enforces the institutional policies outlined in this manual and is responsible for approving any access to the TB BSL-3 laboratory, provided sufficient training has been completed. The Director must approve of all major changes to operations or procedures in the facility.

Principal Investigator – The Principal Investigator is responsible for ensuring that their research staff has received sufficient training for work in the facility, and for ensuring that their staff follows the procedures outlined in this manual.

BSL-3 Lab Trainer – The BSL-3 Trainer or his/her alternate is appointed by the BSL-3 Director and is responsible for the direct training on entry/exit procedures and all operational and facility rules for those entering the facility. The BSL-3 Trainer is also the key contact for support staff (Facilities, EHS, etc.) that may require occasional entry into the facility under supervision.

Research and Support Staff – Each researcher (PI, Post-doc, Graduate Student, etc.) or support staff (Facilities, EHS, etc.) must participate in required training, occupational health, and other requirements to adhere to the procedures outlined in this manual. Disregard or neglect of documented requirements or PPE use will be treated as a serious offense and can result in suspension from ability to work in the TB BSL-3 Laboratory.

Institutional Biosafety Committee (IBC) – In accordance with the CDC and NIH Guidelines, the IBC is charged with reviewing and approving all work with infectious agents, recombinant DNA, operations, and procedures in the TB BSL-3 Laboratory. The committee members include the Biosafety Officer, Facilities Director, EHS Manager, and various Principal Investigators and non-affiliated community members. Members of the IBC must perform an inspection of the facility annually.

Environmental Health & Safety (EHS) – Environmental Health & Safety Personnel assist the IBC in review of operations and procedures to ensure compliance with the Washington State regulations concerning agents that can be transmitted via the bloodborne route. EHS also assists the IBC in ensuring compliance with CDC and NIH Guidelines. The EHS Department also performs quarterly inspections of the facility with the Safety Committee, tracks training compliance and reports any safety concerns to the BSL-3 Director.

Facilities Department – The Facilities Manager is responsible for completing BSL-3 facility maintenance documentation records and verifying facility-related operations (i.e. HVAC, alarm systems, filtration replacements) annually, or at intervals specified in the BSL-3 Annual Maintenance Certification Document maintained by Facilities Department.

B. Containment Levels for TB Research

The BSL-3 designation typically applies to research and production facilities in which work is performed with agents which may cause serious or potentially lethal disease. The Center's TB BSL-3 Facility operates as a BSL-3 for typical TB laboratory-scale research. The bacterial pathogens *Mycobacterium tuberculosis* and *M. bovis* are classified by the NIH and Centers for Disease Control and Prevention (CDC).

Biosafety Level 2: The use of recombinant TB DNA containing less than two-thirds of the genome, full-length TB DNA in *E. coli*, or as free DNA can be conducted in an approved laboratory with a minimum of Biosafety Level 2 facilities, practices, and procedures.

Biosafety Level 3 – The Center meets or exceeds CDC, NIH and Washington State regulations by requiring all handling of live TB cultures or samples that may contain the bacterium in the TB BSL-3 Facility using BSL-3 practices and procedures. Introductions of full-length TB DNA into eukaryotic cells must also be performed in in the TB BSL-3 Laboratory using BSL-3 procedures.

C. Regulatory Requirements for TB Research

Multiple regulatory agencies and federal entities mandate performance of a risk assessment of biological research activities and consequent implementation of controls to mitigate risk to research staff, support staff, and the general public.

CDC – The CDC Publication, *Biosafety in Microbiological and Biomedical Laboratories (5th Edition)* defines the biosafety levels and outlines practices, containment equipment, facilities and requirements for performing work with TB and *M. bovis*. Agent summary statements on TB and other agents are provided to assist institutions in meeting the requirements. The Center is required to follow the requirements in this publication due to its receipt of Federal grant funding. A verbatim copy of the BSL-3 requirements from this publication is appended within this manual.

NIH – The NIH Office of Biotechnology Activities (OBA) publishes the NIH Guidelines, of which The Center is required to follow due to its receipt of Federal grant funding. The rDNA Guidelines specify requirements for rDNA research involving infectious agents and outlines containment requirements.

State of Washington –The State of Washington also has enacted multiple general safety, chemical, biological, and hazardous waste regulations with which The Center must comply. The Center's Safety Manual is intended to address and comply with such applicable regulations.

King County & City of Seattle – The handling, treatment and disposal of Biohazardous Waste is regulated by the King County Board of Health under the authority of the Seattle Municipal Code 21.43.030 and Title 10.07.060 of the King County Solid Waste Regulations.

D. Authorization for Research Activities, Inspections and Annual Review

Use of new agents or procedures in the TB BSL-3 laboratory requires prior BSL-3 Laboratory Director approval, IBC approval, and an approved addendum to these procedures. IBC applications for new agents or procedures must explicitly address how the researchers, including those not working directly with the new pathogen, will be protected from exposure to the new agent. Protocols must be outlined in the context of the TB BSL-3 laboratory. If so approved, the facility may be used for work with multiple organisms.

If experiments involving other organisms or work are to be conducted in the BSL-3 facility that require lower levels of containment, they shall be conducted in accordance with all BSL-3 practices and procedures outlined in this manual. All such work must receive approval prior to work start as described above.

This manual and its procedures are reviewed on an annual basis and modified as needed by the BSL-3 Director or his/her designate, Environmental Health and Safety Manager, and the Institutional Biosafety Officer, in consultation with all PI's using the BSL-3 Facility. Maintenance and facility-related requirements are to be completed and documented on an annual basis by the Facilities Manager and his staff, in accordance with the BSL-3 Annual Maintenance Certification Document. To ensure overall compliance, designated and trained members of the Institutional Biosafety Committee and The Center's Internal Safety Committee perform inspections of the BSL-3 facilities at least annually. The Biosafety Officer inspects the facility at least annually. Inspections are documented on the BSL-3 Lab Safety Inspection Form and maintained by EHS.

E. Training and Access Requirements

Entry Program Components

The TB BSL-3 Laboratory Director or his/her designate must be consulted whenever new persons desire entry into the BSL-3 laboratory. With input from the Principal Investigator, Biosafety Officer and EHS Manager, the BSL-3 Director has the final responsibility for determining who may enter or work in the laboratory. All persons entering the laboratory must have prior approval from the BSL-3 Laboratory Director and fulfill all other requirements for entry into the BSL-3 facility. Children under age 18 are not permitted to enter the laboratory under any circumstances. Entry of persons who refuse to comply with all entry procedures will not be allowed. Access to the laboratory is restricted to persons whose presence is required for program purposes: (a) Research personnel performing operations approved by the IBC; (b) Maintenance and repair personnel; and (c) Official observers and Inspectors.

Before entering, the specific biohazards present in the BSL-3 facility are described, and each person receives applicable training in BSL-3 procedures. This training will be provided by the Principal Investigator if he or she is experienced in BSL-3 procedures, or training will be conducted by the BSL-3 Trainer designated by the BSL-3 Director. Training records are maintained by EHS in the staff member's EHS training file. EHS provides staff with all necessary occupational health offers, such as TB testing. Paperwork verifying these offers are maintained by EHS. All medical records are maintained by the healthcare provider. All entry into the facility must be pre-approved by the BSL-3 Director.

The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. This training must be documented (See
Figure 2. TB BSL-3 Training Documentation). Personnel must receive annual bloodborne pathogen training, annual respirator training, and additional training when procedural or policy changes occur.

Occupational Health Requirements
TB Testing or an approved monitoring method outlined in the EHS document, "TB Testing Program and Policy" is required prior to any entry by Center staff into the facility. Most testing is required on a semi-annual basis thereafter, or as medically indicated. Record keeping is confidential and follows The Center's Safety Manual.

Respiratory protection is required for any entry into the BSL-3. All intending to enter are required to enroll and participate in The Center's Respiratory Protection Program. This includes receiving respirator medical clearance from a healthcare professional, respirator fit testing and training. Contact EHS to enroll in this program.

Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations, prophylactic interventions or treatments. Therefore, all laboratory personnel are provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Entry for Research Personnel
Research personnel must complete the standard Center for Infectious Disease Research Safety Training provided by EHS, review this manual, complete the TB BSL-3 test, complete all required occupational health programs, meet with the BSL-3 Director, and receive in-lab BSL-3 training from the BSL-3 lab trainer. Research personnel will enter the facility the first time accompanied by the PI or BSL-3 Trainer for at least one day of observation of experienced workers in action. Next, the trainee will go through at least 2 days of hands-on work with direct supervision by the trainer or other experienced BSL-3 laboratory personnel. When proficiency is demonstrated, the BSL-3 Trainer, PI and BSL-3 Director approve and the trainee is allowed to enter the BSL-3 facility and perform experiments unsupervised. However, the PI may elect to observe the new worker before granting approval for keycard access and entry alone.

Entry for Facilities Personnel & Internal Inspection Personnel
Facilities personnel must complete the standard Center for Infectious Disease Research Safety Training provided by EHS, review this manual, complete the TB BSL-3 test, complete all required occupational health programs, and receive BSL-3 training from the BSL-3 lab trainer. Facilities personnel must enter the facility accompanied by an experienced trainer as outlined above. The trainer will judge when the employee has become fully acquainted with BSL-3 procedures for future entry, accompanied by approved TB BSL-3 laboratory staff. Facilities personnel may only enter alone in the case of an emergency, with permission from the TB BSL-3 Director. In addition, they will receive information about specific biohazards associated with the equipment to be repaired or examined. Some facilities personnel will be designated to enter the facility alone following successful completion of training. Other facilities staff will only be allowed to enter when accompanied by an approved staff member, the BSL-3 Trainer, or another qualified individual designated by the BSL-3 Director. Facilities personnel may enter the TB BSL-3 facility only when no active work is being performed (i.e. no TB cultures outside of incubators).

Entry for Special Visitors (not performing work)
Special Visitors (not performing work) shall be allowed entry only in highly unusual circumstances (i.e. visiting TB PI), and must be approved by the BSL-3 Director and V.P. of Operations and Finance. The EHS Manager and Biosafety Officer shall be notified prior to visitor entry into the BSL-3 laboratory. Visitors shall be accompanied at all times by the BSL-3 Director or an experienced person designated by the Director. Visits may occur only after at least one full overnight air exchange and prior to any infectious

work beginning for the day. No infectious work is to occur during the visit. Visitors shall be required to follow The Center's standard BSL-3 entry and exit procedures, including use of required Personal Protective Equipment (PPE) including gowning, N95 respirators, and other PPE. Due to regulatory constraints, visitors are not permitted to wear PAPR's and thus will not be allowed access to any PAPR-required area, as defined in the TB BSL-3 Respirator Use Table (Table 1). Visitors shall be allowed entry only upon completion of a signed acknowledgement indicating their understanding of the following notifications, and agreement with the stipulations of The Center's program as outlined below.

1. The visitor must attest, in writing, that their institution maintains a comparable TB safety training program.
2. The visit shall occur after at least one full overnight air exchange, and before any infectious work occurs for the day.
3. The visitor shall be required to follow The Center's standard entry and exit procedures, including use of required PPE.
4. The visitor is responsible for ensuring proper fit of the PPE that The Center will provide.
5. The visitor agrees that they will be entering the BSL-3 laboratory at their own risk, and that The Center shall not be liable for any injury or damages caused by their visit, except to the extent caused by the sole negligence of The Center.

Security & Keycard Access

The facility uses additional security features by employing keycard access to authorized and trained personnel only. Keycard access for any person entering the facility is requested and approved by the TB BSL-3 Laboratory Director only. EHS may provide status of training and occupational health to the BSL-3 Director to assist him/her in choosing who is cleared for keycard access. The Director of Facilities and Operations, or Facilities Manager, will update keycard access accordingly once communication to do so is received from the BSL-3 Director.

II. BSL-3 Facilities, Equipment, Procedures and Standard Microbiological Practices

A. Facility Description & Engineering Controls

The Facility and Access Control

The TB BSL-3 laboratory is located at the North end of the 2nd floor in the 307 Westlake building. The BSL-3 laboratory is separated from the BSL-2 laboratory corridors by an anteroom. Personnel must enter through 2 sets of self-closing doors from access corridors. Doors are interlocked so that only one door may be opened at a time, and the outer access door must remain locked at all times. Internal laboratory doors between the BSL-3 Laboratory Suites and the Equipment Corridor are kept closed at all times.

Entry to the BSL-3 laboratory is by key-card. Access to the laboratory is restricted to persons whose presence is required for program purposes. Qualified personnel will be issued access at the discretion of the BSL-3 Laboratory Director after completion of training

The TB BSL-3 laboratory consists of the anteroom, the equipment corridor, and the inner laboratory suites where biosafety cabinets, centrifuges, and microscopes are located and work is performed. The equipment corridor used for autoclaving, incubation of non-liquid samples, equipment and stored samples only. No experimentation shall be performed in the equipment corridor.

Signage

A hazard warning sign, incorporating the universal biohazard symbol, is posted on the laboratory access doors. The hazard warning sign identifies any special requirements for entering the laboratory including

personal protective equipment, the organism(s) under study and person(s) responsible for the laboratory. In addition, refrigerators, freezers, biohazard waste receptacles, and other containments shall be labeled with the infectious agent or substance and the universal biohazard symbol. Signage is updated when there is turnover of emergency contact staff, infectious agents used, or change in entry procedures/PPE, and also reviewed for accuracy / updated as needed during the routine inspections.

Ducted Air-Exhaust System & Directional Airflow

A ducted exhaust air ventilation system is provided. This system creates directional air flow that draws air into the laboratory from the BSL-2 laboratory areas through the entry area and into the BSL-3 facility. The exhaust air is not recirculated to any other area of the building. All air from the facility is HEPA-filtered, and discharged to the outside, away from air intake vents. HEPA filters for ducted air exhaust are monitored with Magnehelic gauges, and are checked periodically for filter loading, and changed before directional airflow is impacted. The HEPA-filtered exhaust from the class II biological safety cabinets discharges into the same exhaust system ducts as the BSL-3 room air.

Air Pressure Monitors (APM's)

The ducted exhaust air ventilation system creates directional airflow and maintains negative pressure gradients across each of the doors that lead into the facility. The pressure decreases in a stepwise gradient from the general BSL-2 laboratory, through the anteroom and BSL-3 equipment corridor, and into the BSL-3 laboratory suites.

Personnel must verify the airflow each time the laboratory is entered using the outermost APM's. A negative pressure differential of -0.03 inches of water or less (-0.05, -0.09, -0.1, etc) must be maintained in order to ensure integrity of containment. If pressure is more positive than -0.03 inches of water (-0.02, -0.01, or greater), contact the Facilities Department and do not enter the BSL-3. If pressure is excessively negative at more than -0.1 inches of water (i.e. -0.11, -0.15, -0.20), leave the facility and return when pressure returns to standard. Personnel working inside of the BSL-3 must also be notified to immediately cease work until the Facilities Department can investigate and ensure that containment can be restored.

Biosafety Cabinets (BSC)

BSCs are required to be located away from areas of travel or flow. They are certified when moved, installed, and annually by trained, qualified technicians from a third-party contractor. The HEPA-filtered exhaust from the class II biological safety cabinets discharges into the same exhaust system ducts as the BSL-3 room air. A detailed description of BSC operation is included in the Equipment Section of this manual.

Vacuum Line Protection

CDC and NIH require that vacuum lines be protected with liquid disinfectant traps and high-efficiency particulate air (HEPA) filters to filter of the same or greater efficiency. Filters must be replaced after 180 days of use or if wetted or noticeably blocked, or replaced as necessary.

Cleanability

All surfaces (doors, walls, floors, and ceilings) are constructed to facilitate easy cleaning and decontamination, ideally with water-resistant surfaces; bench tops are impervious to water, and resistant to organic solvents, acids, alkalis, and chemicals used for cleaning/decontamination. Chairs and other furniture must be constructed of non-porous materials that are easily cleaned with disinfectants. Floors are slip-resistant and cracks or openings are sealed.

Sinks

Sinks for both laboratory use and hand washing are provided throughout the facility. Operational hardware for sinks located within the TB BSL-3 Suites are considered hands-free, and are to be operated using the

forearms. In the unlikely event that gloves are removed while working in the Suites, forearm-operated paddles are provided to facilitate hands-free sink operation. A sink is also provided in the Anteroom for hand washing upon exit from the facility. These sinks use automatic motion-detection battery-operated hands-free hardware. Any changes in sink operation efficiency must be reported to the Facilities Department for immediate correction.

Autoclaves

A pass-through autoclave for decontaminating laboratory waste is maintained in the TB BSL-3 facility. All laboratory waste is autoclaved by the research personnel working in the BSL-3. A detailed description of autoclave operation and the sterilization protocol are found in EHS SOP #EHS-004 (available on the intranet). This SOP outlines standard operating procedures for autoclaving of biohazardous waste.

Integrated Pest Management Program

The presence of pests, such as flies and cockroaches, can mechanically transmit disease pathogens and compromise the research environment. Therefore The Center has instituted an Integrated Pest Management (IPM) Program to identify and eliminate pest problems in its BSL-3 Facilities. Due to the design of the BSL-3 facilities at The Center, the risk of a pest infestation is low. To date, no pest infestations have been identified. Design features include HEPA filtered air, negative pressure airflow, and sealed barriers. The primary element of the IPM Program is continuous monitoring whereby personnel are instructed to report the presence of pests in the BSL-3 facilities to the BSL-3 Director and the Facilities Manager. The second element of the IPM Program is housekeeping and maintenance. By maintaining housekeeping and clean equipment in good working order, potential pest habitat is reduced.

In the event of identification of entomological pests in the BSL-3 facility, the BSL-3 Director and Facilities Manager will consult with trained pest management professionals and undertake pest eradication. The BSL-3 Director is required to notify the IBC of any issues that arise so that the IBC may review actions taken to mitigate potential impacts. In the case of an incident that requires pest eradication, this program will be reevaluated for efficacy and modified to further reduce or eliminate future infestations.

B. Entry, Exit Procedures and Personal Protective Equipment Requirements

Entry into the TB BSL-3 laboratory is limited to those individuals who have been trained and authorized as previously described. Those in training must be accompanied by the TB BSL-3 Trainer, PI, or other experienced individual approved by the TB BSL-3 Director. All occupational health programs (contact EHS) must be offered prior to entry into the facility.

TB Testing - TB testing, or chest X-ray and symptoms survey when appropriate, must be completed prior to entering the BSL-3 for the first time and semi-annually thereafter or as medically indicated. TB monitoring follows The Center's "TB Testing Program and Policy," and all test results are confidentially maintained by the EHS Manager.

Respiratory Protection Requirements - Respiratory protection is required all entry into the BSL-3 facility. Contact EHS to enroll in the respirator program. Both N95's and PAPR's are used within the BSl-3 facilities, depending on the type of work being performed. Use of respirators requires that the user be declared medically fit, fit-tested, and properly trained prior to use.

Jewelry - Any jewelry worn on the hands or wrists into the TB BSL-3 must be covered by protective clothing. Rings of a design likely to perforate gloves may not be worn.

Music Players - Personal music playing devices with headphones are not permitted to be used in the BSL-3 facility.

Personal Items - Personal items such as coats, hats, purses, food, water bottles, etc. are not permitted in the laboratory.

Mobile Phones - Talking, texting, taking photos or accessing data on personal mobile phones is not permitted within the facility. A landline telephone and computers are provided in the facility for staff use.

Negative Pressure Airflow Checks

- Prior to entering the anteroom, personnel must perform the following checks:
- Perform a visual inspection verifying that the opposing anteroom door is closed, and
- Verify that the active pressure monitor airflow negative pressure differential is -0.03 inches of water or less (-0.05, -0.09, -0.1, etc) to ensure integrity of containment.

If pressure is more positive than -0.03 inches of water (-0.02, -0.01, or greater), contact the Facilities Department and do not enter the BSL-3. Personnel working inside of the BSL-3 must also be notified to immediately cease work until the Facilities Department can investigate and ensure that containment can be restored.

C. PPE Requirements

A general overview of PPE requirements is provided below. Additional details regarding required PPE depending on location in the facility is also provided.

N95 Respirators

Respiratory protection must be worn each time the user enters the TB BSL-3 facility. N95 respirators are used for low-risk studies and general entry into the facility. Only NIOSH-approved N95 respirators that have been approved by both the TB BSL-3 Director and EHS Manager may be fitted and used. All use follows The Center's Respiratory Protection Program.

Powered Air Purifying Respirators (PAPR's)

Respiratory protection must be worn each time the user enters the TB BSL-3 facility. PAPR's are required to be worn when performing higher-risk studies, cleaning up spills, or entering work areas where such work is in process. Only the 3M Air-Mate PAPR with BE 10 Hood may be used in the facility, and all use follows The Center's Respiratory Protection Program.

Gowning

Workers in the BSL-3 laboratory are required to wear specific protective laboratory clothing. Disposable Tyvek coveralls with a solid-front must be worn. Other gown types must be approved by the Principal Investigator, BSL-3 Lab Director, and Environmental Health & Safety Manager. Protective clothing is not worn outside of the BSL-3 facility.

Eye Protection

Approved eye protection of safety glasses, faceshield, PAPR hood, or goggles shall be worn in the BSL-3 Suites upon entry and at all times.

Shoes and Shoe Covers

Closed-toed shoes are required for all laboratory work at The Center. Shoe covers (booties) are required to be worn upon entry into the facility.

Hairnet

Staff shall wear a hairnet to contain hair so as to prevent the temptation to reposition hair. Staff with longer hair is encouraged to secure the hair with rubber bands or clips to prevent hair from escaping the hairnet.

Gloves

Two pair of gloves (double-gloves) must be worn to protect hands from exposure to hazardous materials. Powdered latex gloves are not allowed in the facility due to risks to those with latex allergies. Nitrile gloves are typically used within the facility. Always follow proper glove removal technique to ensure that outer gloves never touch bare skin. Gloves must not be removed from the BSL-3 facility. In addition, BSL-3 laboratory workers must:

- Always secure outer gloves over Tyvek suiting using tape.
- Change outer gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
- When exiting a Suite to leave the facility after handling of open, live infectious samples, spray outer gloves with an approved disinfectant and remove outer gloves prior to exiting the BSL-3 Suite and moving into the Equipment Corridor.
- If exiting a Suite where no handling of open, live infectious samples has occurred, it is permissible to move between Suites provided outer gloves have been sprayed with an approved disinfectant prior to exiting the BSL-3 Suite. Outer gloves are then removed prior to exiting the facility.
- Upon final exit, remove inner gloves and wash hands in the anteroom before leaving the facility.
- Not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

Minimum Required PPE if Performing Work in the Equipment Corridor Only - Includes checking supply stock or performing maintenance activities.

(Don the following PPE in the Anteroom)

- Tyvek Coverall Suit with Closed Front or Lab coat (lab coat must be autoclaved upon exit)
- 1 pair of gloves
- N95 Respirator
- **Important Note: Entry into the BSL-3 Suites requires additional PPE**

Additional Required PPE for Entry into the BSL-3 Suites

(Don all PPE in the Anteroom. Exception: Don Eye Protection in Equipment Corridor if wearing an N95)

- Shoe covers (booties)
- Tyvek Coverall Suit with Closed Front
- First pair of gloves, secured over the coverall with GloveLock Tape or similar to provide a secure barrier that will not be compromised during regular outer glove changes within the BSL-3 Suites.
- Hairnet
- Respirator (N95 or PAPR as required by the BSL-3 Respirator Use Table and PAPR Operation Checklist)
- Approved Eye Protection (safety glasses, goggles, face shield, or PAPR hood)
- Second pair of gloves

All required PPE must be worn as specified at all times when inside the facility. Double-gloving is required so that the outer pair can be easily discarded and replaced in the event of a known contamination or ripped outer glove.

PPE Exit Requirements

When exiting the BSL-3 Suites, PPE is removed in the following order.

- Outer Gloves are either discarded within the Suite prior to exit or in the Equipment Corridor depending on whether handling of open, live infectious samples has occurred). See the Gloves Section above for additional clarification.
- Reusable Eye Protection is removed in the Equipment Corridor and stored in a lidded plastic container on the benchtop. Disposable Eye Protection is removed in the Equipment Corridor (see below).
- If wearing a PAPR, it must be sprayed with Accel-TB/PREEmpt prior to exiting the BSL-3 Suite.

When exiting the Equipment Corridor, PPE is removed in the following order.

- Tyvek Coverall Suit removed by rolling suit inside-out
- Shoe Covers (Booties)
- Disposable Eye Protection, Hairnet and Respirator (N95 or PAPR following requirements outlined in the PAPR Operation Checklist)
- Outer (if applicable) and Inner second pair of gloves

PPE for disposal is placed inside of designated biohazard waste containers located in the Equipment Corridor. Remove Tyvek suit by rolling the suit inside out during removal. Tyvek suit and Shoe Covers are placed in the biohazard waste autoclave bag located next to the Autoclave. All other PPE (Gloves, Hairnet, Disposal Respirator) are placed in the open garbage bin next to the Equipment Corridor door that exits into the Anteroom. All used PPE is autoclaved prior to removal from the facility. No PPE used at any time within the facility (other than a cleaned PAPR unit) may be removed from the Equipment Corridor into the Anteroom. Hands are washed in the anteroom hands-free automatic sink prior to exit from the facility. The Centers for Disease Control recommend washing hands for at least 20 seconds. For BSL-3 operations, hand sanitizer may not be substituted for hand washing with soap and water.

Re-Use of PPE

Certain types of PPE may be reused, depending on the circumstances. A description of permissible PPE reuses is listed below. Additional PPE reuse scenarios require prior approval from the TB BSL-3 Director and addition to this manual.

- Reusable eye protection, such as safety glasses, shields or goggles may be reused. Such protection may be cleaned with 70% ethanol as needed, and must be stored in a lidded container in the Equipment Corridor when not in use.
- PAPR Hoods and Blower/Filter Units are reused. PAPR hoods are disinfected prior to removal from the facility and stored in lidded containers or drawers within the Anteroom. PAPR Blower/Filter Units are also decontaminated and stored on shelving in the Anteroom during charging.
- Tyvek Coverall Suits may be reused in the following circumstances only:
  a. When the suit has been worn in a Suite where open TB cultures have not been used during entry.
  b. When the suit is expected to be worn again within one week.
  c. Such suits must be labeled with initials and date, rolled up and stored on the shelf by the entrance to the anteroom in a lidded container.

D. Storage of Items in the TB BSL-3

Storage of infectious TB samples must be in screw cap style cryotubes, 15 or 50ml conical tubes inside secondary containment. Snap cap microfuge tubes are not sufficiently leak proof or durable.

E. Removal of Items from the TB BSL-3

All information about experiments performed in the BSL-3 is communicated electronically, via computer linkage, with one-way (into the facility) paper entry.

All items to be removed from the laboratory, including equipment, PPE, waste, and infectious material for shipment must be properly decontaminated or secured. The Decontamination and Transport Sections in this document detail such requirements.

F. Standard Microbiological Procedures & Practices

The following CDC, NIH and WAC 296-82 standard and special safety practices, and internal Center requirements apply to the BSL-3 facility and research.

- All personnel shall follow Universal Precautions while in the laboratory, treating all material as if it were infectious. Always assume that infectious material is present in the laboratory and in/on all equipment and devices that come into direct contact with known infectious materials, unless decontaminated or cleaned,
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption is prohibited in the TB BSL-3 laboratory areas.
- Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- The use of animals is not permitted in the facility. Plants are also not permitted in the facility.
- Paper and laboratory notebooks may only be removed from the facility via autoclaving or chemical disinfectant treatment. Therefore, the experimental data from work is transferred via electronic file or email from the computer located within the TB BSL-3 facility to servers that can be access outside. Reliable computer linkage is critical, thus the IT Department will service linkage losses with high priority.
- Sharps use, including needles, Pasteur pipettes, razor blades and similar items are not permitted in the TB BSL-3 laboratory facilities. Some disposable pipette tips can be considered sharps, and their use should be approved by the BSL-3 Lab Director as needed. Use of glass is discouraged and plasticware is preferred. Some glass culture flasks and chemical bottles are used when plasticware is not sufficient. Occasionally, microscope slides are used for eventual removal from the facility. Slides are fixed with paraformaldehyde, sprayed with Accel-TB/PREEmpt, and then removed for microscopic analysis. Additional details are described in the Decontamination Section.
- All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as aerosol canisters or sealed rotor must be used.
- All procedures must be performed to minimize the creation of splashes and/or aerosols. All pipettes used with mechanical pipetting devices, with the exception of aspirating pipettes, are plugged with cotton or similar material. Pipetting, pouring, vortexing, shaking, and centrifuging all have the potential to generate aerosols.
- No infectious materials may be left out in workspaces overnight.
- For transport within the facility, potentially infectious materials must be placed in durable, leak-proof containers during collection, handling, processing, storage or transport. Such containers must be in secondary containment with exterior sprayed with disinfectant. Always secure lids tightly before transporting items from the BSC. Multi-well dishes must be transported in covered, plastic containers to contain any leaks or spills that could result from the worker being

tripped or jarred during transport activities. For transport of materials outside of the facility, see the Transport of Infectious Material Outside of the Facility Section for additional requirements.

- All cultures, stocks, wastes and other potentially infectious materials must be decontaminated prior to disposal or removal from the facility using an effective method. The section on Decontamination covers this topic in detail.
- Laboratory equipment and the facility itself must be routinely decontaminated, as well as, after spills, splashes, or other potential contamination. Work surfaces of biological safety cabinets are decontaminated when work with infectious material is completed by each person. Additional details are found in the Equipment and Procedures Section of this manual.
- Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material. See the section on Handling Emergencies for additional information.
- Equipment must be decontaminated before repair, maintenance, or removal from the laboratory. The section on Decontamination covers this topic in more detail.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and are treated according to procedures described in the Handling Emergencies Section of this manual.

III. Equipment Procedures & Practices

To ensure personal protection and to prevent contamination, equipment found in the TB BSL-3 facility must be used in accordance with the procedures identified below.

Biological Safety Cabinets

All work involving TB in uncovered containers and procedures with the potential to generate aerosols must be conducted in a biological safety cabinet (BSC). The BSC is the primary containment device in a biological laboratory due to its ability to re-circulate and clean air. Note that Biosafety Cabinets will only protect you, and your samples, when they are used properly.

Setting up the Biological Safety Cabinet (BSC):

Gather and organize all your equipment and materials for convenience and ease of movement before introducing the infectious material. The following checklist is suggested for preparation:

- Spray bottle of 1% LopHene (must be made fresh daily)
- Disposable serological pipettes – plastic
- Disposable plastic pipette tips
- Spray bottle containing 70% ethanol
- Spray bottle containing Accel TB / PREEmpt (for emergency spills/decon)
- Aspiration flasks (when required) containing concentrated LopHene (such that final concentration when full is >1%)
- Autoclave bag placed in 2 liter plastic beaker (for emptied serological pipettes, pipet tips and other dry waste, see below)
- New bench pads must be placed on the work area where active TB work will be performed. Bench pads are then sprayed with 1% LopHene. Make sure that the pads are not interfering with airflow.

Active Work in the BSC

Work surfaces must be decontaminated before and after use. Note that the UV light is not used for cabinet decontamination in the TB BSL-3 Biosafety cabinets.

- Decontaminate the work surface with 1% LopHene prior to use.

- Avoid airflow disruption: Disrupting the laminar airflow can create eddies and escaping currents potentially carrying aerosols outside the cabinet or drawing contaminants inside.
- Keep objects off grills: Do not place anything over the front air intake grill and do not block the rear exhaust grill. Covering these grills compromises the laminar airflow and reduces operator and culture protection. Work should be done at least 6" from the grills.
- No flaming inside the BSC: Flaming is not allowed in the BSC as it disrupts airflow, degrades HEPA filters, and presents a safety risk.
- Control turbulence: Move arms slowly in and out of the cabinet. Place equipment that creates air turbulence in the back third of the cabinet and stop other work while equipment is operating.
- Minimizing aerosol production: Procedures with a high potential for creating infectious aerosols such as vigorous shaking, vortexing, and bead beating are performed in the back third of the BSC. Procedures must be conducted to minimize aerosolization.
- Aspirator operation and maintenance: Where vacuum lines are used, lines are protected with a vacu-guard filter (replaced after 180 days of use or if wetted or noticeably blocked) and two traps; an aspiration collection flask and an overflow collector. Flasks outside of the biosafety cabinet must be placed in secondary containment. Operators must add concentrated LopHene to the aspiration flask so that the final concentration after the flask is full will be >1% LopHene (w/v). For example, this would be ≥ 20 ml of concentrated LopHene for a 2000ml flask.
- After aspirating infectious material, clean the interior of the line by aspirating a suitable volume of 1% LopHene.

Cleanup and Decontamination of the BSC

Cleanup and decontamination of the BSC and equipment used in it is the sole responsibility of the individual working at the BSC. Users must leave Biosafety Cabinets in a clean and sterile condition after use. Solid waste must be prepared for autoclaving. Liquid waste must be chemically decontaminated.

- Equipment used in the BSC must be decontaminated before being removed from the BSC.
- Serological disposable pipettes, disposable labware, flasks, multiwell plates, large tubes, and all other waste, such as wrappers, paper towels and gloves used in the biosafety cabinet are considered to be contaminated and must be autoclaved as biohazardous waste. To prepare biohazardous waste for autoclaving follow the following procedures;
- Expel, aspirate or remove any remaining liquid from pipets, flasks and other containers into a 1% LopHene solution (vacuum flask or beaker, as appropriate).
- Place dry waste in an autoclave bag set in 2 liter plastic beaker.
- When finished working in BSC, the primary autoclave bag must be closed using a twist tie leaving a 1 inch opening to allow for proper autoclaving
- Decontaminate the exterior of the bag with 70% ethanol prior to removal from the BSC.
- Biohazard bags must then be removed from BSC and placed in the larger secondary biohazard waste bag (24" x 36") in the lidded biohazard waste bin.
- Reusable Equipment: Wipe down with 1% Accel TB / PREEmpt before removing from BSC.
- Pipettors: When removing pipettors from the BioSafety Cabinet, decontaminate them with 70% ethanol prior to removing from hood.
- Cabinet interior: After the removal of all materials, wipe all interior surfaces with 1% LopHene, allow for a 10 minute contact time and follow with 70% ethanol remove residue.
- Restock supplies while allowing for sufficient disinfectant contact time.
- Examine the tray under the work surface. Disinfect and clean, as necessary.

TB Culture Work Practices

- Liquid cultures may only be opened in the biosafety cabinet.

- Liquid cultures may only be used in the TB suites.
- Liquid culture stocks in refrigerators and in drawers should be discarded after 8 weeks.
- Plate stocks must be stored in sealed bags, as secondary containment.
- Live cultures must be transported in secondary containment.
- Prior to transporting live cultures, the outside of the secondary container must be disinfected.

Spills Inside of the Biosafety Cabinet

Reference the Emergency Procedures Section for instructions on handling spills that are contained within the biosafety cabinet.

Incubators

- The Forma incubator in Suite 1 is reserved for liquid cultures on plates
- Incubators in the equipment corridor are used to store agar plate cultures

Microscopes

- Tighten caps on flasks of infectious culture before transporting to the microscope.
- Use secondary containment when transporting material to the microscope.
- When approved for use, glass slides may be used. Glass slides are considered "sharps" and extra care must be used when handling glass slides.
- Disinfect the viewing platform of the microscope after each use.
- When using petri dishes containing TB to determine colony forming units (cfu's) the exterior of the petri dish must be disinfected.
- Due to the lack of secondary containment when using the microscope this is considered a high risk operation and must be performed with considerable care.
- Only fixed (treated with 4% formaldehyde, acetone, methanol, etc.) slides sprayed with disinfectant may be removed from the TB BSL-3 facility

Centrifuge Instructions

Centrifugation is an aerosol generating activity. Follow these guidelines when operating centrifuges in the TB BSL-3.

- Solutions being centrifuged will form aerosols if a sample container leaks, cracks, breaks, or if liquid is on the lip of the tube.
- Before centrifuging, eliminate tubes with cracks and chipped rims, and inspect the inside of the trunnion cup for debris that could cause breakage.
- Centrifuge biohazardous materials in leak proof containers (tubes with protective O-rings) or in Aerosolve canisters for the Beckman Allegra X-15R centrifuge.
- Tubes with O-rings must be used in the microcentrifuge.
- Microcentrifuges must be operated with the use of the rotor cap for aerosol containment.
- Always fill and open tubes in a biological safety cabinet.
- Perform all pipetting or aspiration of supernatant in the biological safety cabinet.
- All tubes must be properly balanced to avoid vibration and/or rotor failure.
- If tube leakage or breakage has occurred, spray thoroughly with Accel TB / PREEmpt, close lid and wait for 10 minutes, remove tube carefully, and discard for autoclaving. Aspirate excess media in rotor or carrier including lid. Remove residual disinfectant with 70% ethanol. Rinse rotor with clean water and dry.
- Frequently inspect, clean, and dry the rotor to ensure absence of corrosion or other damage that may lead to the development of cracks.

- Examine rubber O-rings and tube closures for deterioration and keep them lubricated with the material recommended by the manufacturers. Where tubes of different materials are provided (e.g., celluloid, polypropylene, stainless steel), take care to employ the tube closures designed specifically for the type of tube in use.
- Aerosolve canisters must be removed from the centrifuge and may only be opened in a biosafety cabinet.
- If it's discovered that a centrifuge tub leaked during a spin all liquids and containers and rotors are to be treated with concentrated LopHene for 10 minutes.
- After each use, clean the centrifuge with Accel TB / PREEmpt.
- After conducting low temperature spins, leave the centrifuge door open to evaporate condensation.
- Microcentrifuge rotors must be removed and opened in a Biosafety Cabinet if a spill or tube failure is suspected.
- Aerosol canisters must be dry prior to being placed in anodized aluminum buckets.
- Note that Accel TB / PREEmpt, LopHene and other disinfectants will damage anodized aluminum! When using disinfectants on anodized aluminum rotors make sure to limit contact time to minimum required contact times and follow with 70% ethanol to remove residues.

Liquid Culture Rotators and Rollers

When using rotators and rollers, the maximum volume of liquid must be ≤20% of the maximum capacity of the vessel being used.

- Rotator: 10ml in 50ml tubes; 3 ml in 15ml tubes
- Roller: 50ml in 250ml bottles; 100ml in 500ml bottles

Bead Beater

Bead beaters are used in the TB BSL-3 for mechanical cell disruption. Bead Beaters must be used in a BioSafety Cabinet (BSC) to use when processing live TB samples. It has been demonstrated that bead beating does not sterilize samples even after multiple rounds of bead-beating at maximum speed. Additionally, lysing matrix tubes may leak and send aerosolized samples out during bead-beating.

- Use the outlet on the wall, not the ones inside BSC for Bead Beater. Bead Beater requires higher current to operate than the outlets inside BSC.
- Note that samples in Trizol are chemically inactivated and may be processed outside of the BSC.

IV. Transport, Shipment and Waste Disposal

A. Procedures for Transport of Pathogenic Materials Outside of the TB BSL-3 Facility without deactivation of Mtb

All materials infected with or potentially infected with TB or other infectious agents must be double contained while in transit prior to shipment.

- Containers with fixed tissues or fixed samples must be individually sprayed down with Accel TB / PREEmpt -prior to removal from the facility.
- For shipment or transfer outside the building, infectious materials are to be packaged in accordance with IATA and DOT regulations, per 49 CFR. All containers or tubes must be superficially decontaminated with Accel-TB/PREEmpt prior to packaging in the appropriate shipping containers. The shipping containers and contents themselves must also be sprayed down with Accel-TB/PREEmpt prior to removal from the BSL-3 facility. Only trained, certified Infectious Agent Shippers may package such items for shipment. Contact EHS to obtain IATA and DOT

training and certification for proper shipment of hazardous materials. Contact shipping and receiving for the appropriate paperwork and any changes in shipping regulations. EHS may also be contacted for information on CDC and USDA regulatory permits for such shipments.

- Transport of infectious materials within the facility between suites shall follow the instructions in the Standard Microbiological Procedures section.

B. Waste Disposal, Decontamination and /or transport from BSL 3 by deactivation of Mtb

All cultures, stocks, equipment, waste, and other potentially infectious materials must be decontaminated using an effective method before disposal and prior to removal from the BSL-3 facility. The TB BSL-3 facility uses either chemical disinfection and/or autoclaving for such purposes. Note that bleach is not an effective tuberculocide and is not used in the TB BSL-3 facility.

Methods for killing *Mycobacterium tuberculosis*

There are currently three approved ways of sterilizing samples prior to removal of *Mtb* from the BSL-3. Any variation on the protocols below or alternative procedures must first be validated, documented, and discussed with the BSL-3 Director and EHS Manager prior to use.

1. Heat killing

Mtb can be heat-killed by heating to 95°-100°C for 30 min.. For heat-killing, samples must be in screw cap tubes.

- Bring a heating block to the appropriate temperature, check temperature with thermometer
- Place screw cap tube with *Mtb* sample into the block and cover with LopHene-soaked paper towel
- Invert the tubes periodically to mix
- After the appropriate time, turn off heat source and remove sample
- Wipe tube with 1% LopHene and with Accel TB / PREEmpt prior to removal from the BSL3

Note: Limit volume to 1.0 ml in 1.5 ml tube and cell density to OD_{600} of 1.0. Do not use skirted tubes.

2. Sterile filtering

In case that heat killing affects downstream applications, samples such as cell lysate can be filtered for sterilization using spin column filters or syringe filters. Spin column filters are the filtration method of choice for sample sizes of less than 750ul. Both methods must pass samples through 2 filtration steps and the 0.22μm filter MUST be used at least once.

Spin Column Filtering Steps:

- After bead-beating, centrifuge the sample to pellet beads, insoluble material, and intact Mtb (4000g, 10min) or remove supernatant directly from culture of adherent cells (i.e. macrophages).
- In clean BSC, aliquot no more than 750ul supernatant into a 0.22um filtered spin column
- Wipe outside of tube with 1% LopHene prior to removal from the BSC. Transfer spin columns to benchtop centrifuge.
- Centrifuge at 5000xg for 3-5 minutes. If liquid has not completely passed through the filter by that point, the sample can be spun for additional time.
- Return tubes to the BSC and discard filter inserts.
- Transfer liquid to a second filtered spin column and repeat steps 3-5.

- Make sure to discard second filter insert and decontaminate outside surface of the tubes before removing them from the BSC and BSL-3.

Syringe filtering and applying too much pressure can easily cause aerosols, forceful spilling, and/or filter failure. Do not force sample through filter. Always cover the syringe and filter with a LopHene-soaked rag between the BSC opening and the sample.

- After bead-beating, centrifuge the sample to pellet beads, insoluble material, and intact *Mtb* (4000g, 10 min)
- In a clean BSC, pass supernatant through a 0.45 μm syringe-top filter, and then
- Pass supernatant into a new tube through a 0.2 μm syringe-top filter
- Wipe the outside of the tube with 1% LopHene before taking it out of the BSC and with Accel TB / PREEmpt prior to removal from the BSL3

  Note: Bead-beating is not by itself a sterilizing procedure! To prepare culture filtrate, use vacuum filter units according to published protocols (1).

3. Chemical inactivation

Work inside the BSC using a sealed tube. The following reagents may be used to kill *Mtb*:

- LopHene –use 1% final concentration to sterilize *Mtb* sample
- Chloroform: methanol (1:1) – use equal volume to sterilize *Mtb* sample
- 4% formaldehyde or paraformaldehyde– use equal volume to sterilize *Mtb* sample
- After addition of reagent, invert sample several times to mix contents
- Inactivate for 30 min
- Wipe tube with LopHene prior to removal from BSC and with Accel TB / PREEmpt prior to removal from BSL3
- When inactivating *Mtb* on cover slips, cover slips must be submerged and incubated for 30 min

Note: When using 2% formaldehyde or paraformaldehyde, incubate for 1 hour.

Chemical Disinfectants

LopHene, Accel TB / PREEmpt and T.B.Q. solutions are used in the facility as chemical disinfectants. Other EPA-approved tuberculocidal disinfectants that are shown to kill TB and Hepatitis B viruses may also be used if pre-approved by both the BSL-3 Lab Director and EHS Manager.

LopHene (Decon labs)- LopHene is the primary disinfectant used in the TB BSL-3 for sterilizing TB contaminated material. LopHene relies on phenols, detergents and acidity to effectively disinfect.

- LopHene is used at a 1% final concentration
- 1% LopHene solutions are made by adding 1 part concentrated LopHene to 99 parts distilled water.
- Record the date made on the container.
- LopHene solutions must be made daily per the manufacturers specifications.
- 1% LopHene requires a 10 minute contact time to be effective.
- The chemicals in LopHene will damage metal work surfaces and must be removed using a 70% ethanol solution.
- LopHene may not be used with oxidizers such as bleach, which may cause the release of toxic gas.
- Concentrated LopHene has a shelf life of three years from date of manufacture. The expiration date can be found on the product.

Accel TB / PREEmpt (Virox Tech.) - Is very effective against TB, but is significantly more expensive than LopHene. Accel TB / PREEmpt is a hydrogen peroxide based disinfectant. Accel TB / PREEmpt is to be used for spills and other situations that require a "quick" and effective sterilization. Accel TB / PREEmpt may also be used for equipment decontamination as it is less corrosive and leaves no active residue than LopHene.

- Accel TB / PREEmpt is supplied at the effective working concentration and may not be diluted prior to use.
- Accel TB / PREEmpt requires a 5 minute contact time
- Accel TB / PREEmpt is toxic and a strong irritant and contact with skin and eyes is to be avoided.
- Accel TB / PREEmpt has a shelf life of three years from date of manufacture. The expiration date can be found on the product.
- Accel TB / PREEmpt does not leave active residue
- Accel TB / PREEmpt is considered compatible with stainless steel, aluminum and most polymer plastics
- Note that Accel TB / PREEmpt is not recommended for use with anodized aluminum.

70% Ethanol - While somewhat effective against TB, ethanol is not an EPA-approved tuberculocidal agent and is not to be relied on as a sole chemical disinfectant. 70% ethanol is used after chemical disinfection to remove any residue that may damage surfaces or equipment. Additionally, 70% ethanol is used in the TB BSL-3 for general disinfecting and cleaning. 70% ethanol is not to be used for disinfection of TB cultures or known contaminations.

- The recommended contact time for disinfection is 10 minutes.
- 70% ethanol solutions are made by adding three parts water to seven parts 95% ethanol.
- Never substitute methanol for ethanol in disinfecting applications because methanol is not as effective, and more toxic.
- Ethanol is highly flammable and must be kept away from potential sources of ignition.

Bleach Solutions – While not effective against *Mtb,* bleach solutions may be used for human/mammalian cell lines or blood that has **never** been exposed to *Mtb*. If the sample has an unknown *Mtb* status then bleach cannot be used. Bleach is not an EPA-approved tuberculocidal agent and should not be used with any *Mtb* experiments.

- Bleach bottles must be clearly labeled with the following words, "Not for use with *Mtb* involved experiments." Contact EHS for labels for this purpose.
- Concentrated household bleach typically contains between 5-10% of the active ingredient, sodium hypochlorite.
- The concentrate is diluted by adding one part household bleach to nine parts water, creating a 10% bleach solution.
- Fresh 10% household bleach solutions must be prepared daily to ensure adequate sodium hypochlorite to deactivate susceptible pathogens.
- The recommended minimum contact time for disinfection of bloodborne pathogens such as HIV, Hepatitis B and Hepatitis C is at least 10 minutes.
- Bottles of concentrated bleach can also lose effectiveness, and should be discarded 6-months after initial opening.
- All bleached items, such as plasticware or serological pipettes, are rinsed with water prior to placing them in autoclave bags.

- Bleach solutions are classified as irritating and corrosive to skin and tissues. Chlorine solutions should **never** be mixed with other cleaning products or hazardous chemical wastes. Combining these chemicals may result in the release of a chlorine gas, which can cause nausea, eye irritation, tearing, headache, and shortness of breath.

Autoclaving

All solid waste generated in the TB BSL-3 is considered biohazardous wastes per Federal, State, and Local regulations and must be treated by steam sterilization (autoclaving) prior to disposal. The EHS SOP #EHS-004 outlines the standard operating procedure for the operation of autoclaves and the treatment of biohazardous waste.

No hazardous chemicals, radioactive materials, or any type of equipment (electronic or otherwise) may be run through the autoclave process, as dangerous fumes can be released. Liquid disinfectants should not be autoclaved. Autoclaving liquid disinfectants may produce corrosive, irritating and toxic vapors. Vapors may be released upon opening of the autoclave and will reduce the life of the autoclave through corrosion.

Biohazard waste being stored in the equipment corridor is decontaminated using the pass-through autoclave by an approved and trained autoclave operator. All research personnel using the TB BSL-3 will be responsible for operation of the autoclave. Research personnel individuals will receive training from EHS and/or the BSL-3 trainer on SOP# EHS-004, SOP for the Operation of Autoclaves for the Treatment of Biohazardous Waste.

Waste is autoclaved following SOP# EHS-004. Approved and trained autoclave operators shall wear all appropriate PPE. Biological indicator tests (spore tests) using Bacillus stearothermophilus are loaded weekly. Chemical temperature indicator strips are run with each load. Both Chemical temperature indicator strips and Spore tests are placed near the center of each load. To avoid potential contamination, do not open biohazard bags to place indicators, but rather "sandwich" indicators between closed bags. Records for treatment of biohazardous waste are completed according to the SOP, and maintained for a period of three years in the appropriate records binder for review during inspections and audits. TB BSL-3 staff, EHS and regulators may request a review of these records at any time.

Upon completion of the autoclave cycle, it is the responsibility of the approved and trained TB BSL-3 lab staff autoclave operator to remove the treated waste, check the temperature recorded on the autoclave run to ensure that the autoclave reached a temperature of greater than 120C (250°F) for a period of at least 30 minutes. Information on the run is then recorded in the appropriate records binder. When temperature and time requirements are confirmed, the autoclaved waste is placed into the bin located in the clean-side autoclave room outside of the TB BSL-3 Facility. The Facilities Department staff then removes the autoclaved waste from the bin and transport it to the 1st floor for a second and final autoclave run before eventually placing the treated waste in the standard building trash. If the load did not reach the required temperature, re-autoclave the load in accordance with SOP #EHS-004.

The BSL-3 autoclave is not to be used for general sterilization for the BSL-2 laboratories except under unusual circumstances that have been pre-approved by the Facilities, Director and the BSL-3 Director. In the event of a TB BSL-3 autoclave failure, contact the Facilities Manager immediately.

Below is a summary of guidelines for preparing waste for autoclaving;

- Each person working in the BSL-3 lab is responsible for cleaning up his/her work site and moving lab waste that is to be autoclaved into the equipment corridor.
- Approved biohazard waste bags must be used (supplied by Facilities), and be labeled with a Biohazard Waste label.

- All BSL-3 TB biohazard waste must be double-bagged prior to autoclaving.
- Biohazard waste bags must be kept in an upright container when in use at the work station.
- The primary biohazard waste bag should be filled only 70% full and then prepared for collection.
- Leave 30% of the volume of the secondary collection bag to ensure adequate room for closing.
- Bags are to be "scrunched" closed, leaving a visible opening, and sealed with autoclave tape to allow for the release of steam. Note that "twisting" the bags may result in bursting during autoclaving, resulting in an unsafe condition and may limit the efficacy of steam treatment.
- In the TB BSL-3 all waste is considered to be contaminated with TB and does not require additional labeling.
- Closed and labeled primary biohazardous waste bags are to be placed in the larger secondary biohazard waste bag (24"x36") in the lidded biohazard waste bin.
- Lidded biohazardous waste containers must remain closed when not in use.
- It is the responsibility of each person using the lab to move their biohazard waste bags when 70% full to the autoclave when their work is complete, and at least daily.
- Clean gloves must be used to transport the biohazardous waste outside of TB work suites.
- Used PPE is placed in an autoclave bag in the equipment corridor. Each user is responsible sealing, replacing and preparing use PPE waste for autoclaving when the biohazard bag is70% full.
- Live biohazardous waste must not be stored on the floor.

Facility Surfaces and Equipment

Surfaces and equipment are decontaminated using 1% LopHene followed by 70% ethanol wipe to remove disinfectant residue. Decontamination of the entire laboratory is possible should there be a major contamination event in the space or a major renovation or construction project. In such a case, an outside contractor may be used. Selection of the disinfectants to be used shall be decided on a case-by-case basis.

Mopping

Mopping of the floor with 1% LopHene is to be performed regularly by BSL-3 laboratory personnel. Mopping with LopHene must be followed by a mopping with 70% ethanol to remove residue.

Equipment Removal

If a piece of equipment is to be removed from the BSL-3 space, the following procedures must be followed. Steps 1 and 2 must be performed wearing full TB BSL-3 PPE. For equipment with special considerations, contact EHS for assistance.

1. While in the TB BSL-3 Suites (256C, 256D, 256E), decontaminate the inside of the equipment with an approved, appropriate disinfectant and contact time. Then decontaminate the exterior surfaces of the equipment with 70% ethanol spray, using a contact time of 10 minutes.
2. Move the equipment into the BSL-3 Equipment Corridor (256A), and decontaminate the exterior surfaces of the equipment again with 70% ethanol using a contact time of 10 minutes.
3. After completing the above steps, follow all appropriate procedures for degowning and exiting the TB BSL-3.
4. Move the equipment into the BSL-3 Anteroom (256), performing one last spray with 70% ethanol before taking the equipment from the anteroom into the BSL-2 laboratories.

Aspirator Operation and Maintenance

Vacuum lines are protected with a vacu-guard filter (replaced after 180 days of use or if wetted or noticeably blocked) and two traps; an aspiration collection flask and an overflow collector, both in secondary containment. Operators must add concentrated LopHene to the aspiration flask so that the

final concentration after the flask is full will be >1% LopHene (w/v). For example, this would be ≥ 20 ml of concentrated LopHene for a 2000ml flask.

Sharps Disposal

Most sharps use, including needles, Pasteur pipettes, razor blades and similar items are not permitted in the TB BSL-3 laboratory facilities.

- Some disposable pipette tips may be considered sharps, and their use should be approved by the BSL-3 Lab Director as needed.
- Use of glass is strongly discouraged and plasticware is preferred. Some glass culture flasks and chemical bottles are used when plasticware is not sufficient.
- Blunted surgical scissors and instruments for manipulating tissue are the only exceptions.
- In cases where broken glass or other sharp object requires disposal, the sharp object shall be placed in an approved, red, plastic hard-walled container for disposal. Occasionally, microscope slides are used for eventual removal from the facility.
- Glass slides are considered "sharps" and must be handled carefully.
- Slides that are fixed with paraformaldehyde, sprayed with Accel-TB/PREEmpt, may be removed for microscopic analysis.

Radioactive Waste

The Radiation Safety Officer and State of Washington Division of Radiation Protection must approve any use of radioisotope on a per experiment basis prior to introduction into the BSL-3. Procedures for handling of radioactive waste are determined on a case-by-case basis, prior to beginning the experiment, in consultation with the Radiation Safety Officer. Radioactive waste shall never be autoclaved.

Hazardous Chemical Waste

Hazardous waste is collected in bottles or containers and labeled and handled in accordance with all Safety Manual requirements. Hazardous waste may only be removed from the facility when it can be verified that the waste itself has deactivated all TB or other infectious agents used in the facility. Contact EHS for guidance on approved deactivation methods (use of bleach is not permitted). The outside of the bottle must be wiped down thoroughly with Accel-TB/PREEmpt prior to removal from the facility for hazardous waste pick-up by EHS.

V. Emergency Procedures

Handling Occupational Exposures

An occupational exposure in the TB BSL-3 is defined as any situation where respiratory protection is compromised, resulting in inhalation of non-HEPA filtered air or known to be aerosolized TB. In addition, percutaneous injuries (e.g. cut with a sharp object), contact of mucous membrane or non-intact skin (e.g. exposed eyes or skin that is chapped, abraded, or afflicted with dermatitis), with blood, tissue, infectious TB, or other biological materials that are potentially infectious is considered occupational exposure. Exposure risks are not limited to TB. *Mycobacterium bovis*, HIV, or occasional use of human patient samples that could contain Hepatitis B, Hepatitis C, Herpes B, Epstein-Barr Virus and other infectious agents may be present.

In the event of a potential inhalation, cut, scrape, mucosal contact or other exposure follow these steps:

1. Flush the area with water. If eyes or other mucous membranes are affected, flush with water for a minimum of 15 minutes. If skin is affected, wash with soap and water. Bandage the affected area if needed. If blood loss is substantial, seek immediate medical attention.

2. Immediately notify your Supervisor and EHS. EHS will then notify the Biosafety Officer. Reference the Emergency Flip Chart or Exposure Response Envelope for current contact information.
3. If none of the above individuals are directly available, seek medical evaluation and treatment. Instructions and clinic recommendations are found in the Exposure Response Envelope. Continue attempting to contact your Supervisor and an EHS staff member while en route to the medical clinic. Follow the instructions in the Exposure Response Envelope (posted in the BSL-3 lab and also in the anteroom) and use the taxi vouchers found within the envelope to seek medical care. If human blood, tissues, or blood products were involved, seek medical treatment immediately as administration of antiviral prophylactic medications must begin within 4 hours of exposure.
4. Depending on the type of exposure, a TB test or blood sample may be drawn immediately following the incident so that baseline lab tests can be performed. Follow-up testing may also be required at intervals determined by the medical professional. All information regarding medical records shall be kept confidential and in accordance with WAC 296-802. The Center provides this medical care free of charge to the employee as a part of its Washington State Workers' Compensation insurance with initiation of a claim by completing a Department of Labor and Industries claim form. Additional information regarding confidentiality and occupational exposures can be found in the Emergencies Section of The Center's Safety Manual.
5. Fill out an incident report and return it to EHS within 24 hours of the incident.

Spills Inside of the Biosafety Cabinet

Absorbent pads are used for all work with infectious materials in biosafety cabinets. In the event of a spill, saturate the absorbent pad with 1% LopHene solution. Then cover the spill with paper towels that are soaked with 1% LopHene solution. When work in the cabinet is completed, gather the pads and towels and dispose of as contaminated materials. Ensure that the disinfectant has sufficiently evaporated prior to autoclaving.

All items within the cabinet are assumed to be "contaminated" after a spill, and must be decontaminated appropriately. Spray the insides of the biosafety cabinet thoroughly with Accel-TB/PREEmpt. If a large amount of fluid has spread (>100mL) off of the absorbent pad into the grill and tray, flood the grill and tray with concentrated LopHene and wait 10 minutes. Mop up the disinfectant with absorbent paper or remove the tray and drain it into the sink. Follow with a complete 70% ethanol rinse. It is very important to rinse all metal surfaces with water and 70% ethanol after using LopHene solution to prevent damage to the cabinet and its components.

Spills Outside of the Biosafety Cabinet - A spill of infectious material outside of the biosafety cabinet is a serious situation. In the case of a spill, perform the following steps in order:

1. **Evacuate the Suite and Notify Coworkers:** Tell everyone in the suite that a spill has occurred and they need to exit. Exit the Suite to the equipment corridor. If applicable, remember to hold your breath to reduce the risk of inhalation. Avoid further contamination.
2. **Personal Decontamination:** If personnel are contaminated, spray outer garments with Accel-TB/PREEmpt solution when exiting the Suite. If contamination is significant (i.e. culture spilled directly onto PPE), remove PPE to an autoclave bag after exiting the suite to the equipment corridor. Once in the Equipment Corridor, inspect personal clothing for evidence of contamination. Spray affected personal clothing with Accel-TB/PREEmpt and remove affected clothing. Proceed to the Anteroom and wash hands, or any other potentially contaminated areas using disinfectant soap.
3. **Call/Inform:** Notify personnel in the other suites about the spill. Use phone or other method of communication.

4. **Post Notification Signage:** Place signs on both the door of the suite and the equipment corridor entrance door that reads "Spill occurred in _____(write location)_____ . Do not enter without PAPR!"
5. **Exit the BSL-3 Area:** Remove PPE using the standard sequence outlined in the entry/exit procedures, and exit the BSL-3.
6. **Additional Notification:** Notify the BSL-3 Director, your Principal Investigator and EHS. These people will ensure that the Biosafety Officer is also notified.
7. **Occupational Exposures:** If a potential exposure to infectious material was involved, obtain the Exposure Response Envelope and seek medical treatment immediately following instructions.
8. **Wait:** Aerosols must be allowed to settle [2-3 hours] before proceeding
9. **Clean up:** Perform the following steps to clean up the spill;
    a. Identify 1-2 additional TB-BSL3 trained individuals to assist with spill clean-up.
    b. Identify all required spill clean-up materials.
    c. Don full PPE. A PAPR is required for spill clean-up operations.
    d. Enter the TB-BSL3 facility and begin clean-up.
    e. The equipment corridor must be decontaminated first, followed by decontamination of the affected suite.
    f. Using a squirt bottle maintained for the purpose, prepare a fresh 1% solution of LopHene solution, and lay out a ring of the solution around the outside of the spill area, including remote spatters. Working from the periphery of the spill inward, the spilled material should be covered with paper towels which are soaked with LopHene. This should continue in successively smaller circles until the entire spill is covered with LopHene -soaked towels.
    g. After the required 20 minute contact period, the towels can be picked up and discarded. If the towels carry a large volume of LopHene, it may be necessary to allow evaporate off the disinfectant in the biosafety cabinet prior to autoclaving the towels. Residual LopHene on the cleaned surface must be completely removed as described earlier using a 70% ethanol solution.
    h. Disinfect and discard all clean-up material, as appropriate.
10. **Return to Normal Operation:**
    a. Notify the BSL-3 Director, your Principal Investigator, EHS and TB-BSL-3 users that spill clean-up is complete.
    b. Remove posted signage.
    c. Restock any used supplies.
    d. Complete Accident/Incident Report and return it to EHS within 24 hours.

Equipment Failure

Certain pieces of equipment, such as centrifuges, the bead beater, electroporator, and fermentor present a higher risk of release of infectious material in the event of an equipment failure. If upon entry into, or working within, a BSL-3 Suite, any piece of equipment shows signs of failure (unexpected noise, visible failure or breach, etc.), personnel must immediately turn off the equipment (if possible), exit the BSL-3 laboratory, and follow the procedure for spills outside of the biosafety cabinet as described above.

Emergency Equipment and Contact Information

- An eyewash and safety shower station is located in the Equipment Corridor. Eyewash stations shall be flushed weekly by TB BSL-3 laboratory personnel. The safety shower must be flushed on a quarterly basis. Records of eyewash and safety shower testing are documented by initial and date on the sheet located at the station.
- A fire extinguisher is located within the BSL-3 Equipment Corridor. BSL-3 personnel must familiarize themselves with the location and proper use of the extinguisher.

- A telephone is located on the wall in the TB BSL-3 Equipment Corridor, and in each of the TB BSL-3 Suites. Emergency telephone numbers are posted on the Emergency Flip Chart and found in the Exposure Response Envelopes. Emergency telephone numbers are also found on the entrance door to the facility. EHS updates these materials at least annually, or when a change in procedure or personnel occurs.

Work-Related Injuries or Occupational Exposures

Any injuries or accidents must be reported to your Supervisor and EHS staff immediately. EHS will then ensure that the appropriate Safety Officer and the TB BSL-3 Laboratory Director are notified. An incident report must be submitted to EHS within 24 hours. Instructions for handling occupational exposures are included above.

Medical Emergencies and Door Interlock Release

The emergency door interlock release button outside of the BSL-3 can be used by anyone in the event of an emergency in which a door is blocked. Using this button will activate an institute alarm, and must only be used in planned testing or actual medical emergencies. The button disengages the interlocked door system, but does not unlock the doors. Authorized personnel, including the Fire Department, Security, approved Facilities staff and TB BSL-3 staff can open the door via keycard.

Emergency Exit from the BSL-3

In an emergency such as fire or earthquake, the safety of the researcher is vital. In the case of an emergency located inside or outside of the BSL-3 facility (fire, earthquake, floor, power outage, etc.), immediately close and secure all open cultures. When it is safe to do so, proceed to the exit and observe standard PPE doffing procedures. In the interest of time, PAPR units may be left in the Equipment Corridor without completion of decontamination procedures. Proper storage, decon and clean-up can be completed at a later time after the emergency has passed. After the emergency situation is cleared, don new PPE and re-enter the TB BSL-3 to assess the situation.

Table 1. TB BSL-3 Respirator Use Table

Process/Activity	***Minimum Required Protection***
Entry into BSL-3 (initial minimum requirement)	N95
Decontamination activities in BSL-3 equipment corridor	N95
Work with fermentor	PAPR
Streaking plates in biosafety cabinet, picking colonies (non-aerosolization activities)	N95
All work with MDR TB	PAPR
Work with clinical isolates of unknown drug resistance	PAPR
Work with unfixed lung tissue samples (animal and human)	PAPR
Use of bead beater (in high-containment room)	N95
Work with liquid cultures greater than 300 mL	PAPR
Gas experiments w/ liquid cultures, performed in incubators	N95
Entry into ABSL-3 anteroom	N95
All work in ABSL-3	Reference ABSL-3 Manual
Spill response (in BSL-3 and ABSL-3)	PAPR
Facilities staff entries for repairs, routine maintenance, etc.	N95

* Any entry into the facility requires prior completion of respirator medical clearance and fit testing, and TB testing.

Table 2. Flow Core BSL-3 Respirator Use Table

Process/Activity	***Minimum Required Protection***
Entry into BSL-3 Flow Core and/or Equipment Corridor, if greater than 2 hours since TB, HIV, LCMV or *P. falciparum* (PF) sorting	N95
Entry into BSL-3 Flow Core if less than 2 hours since TB, HIV, LCMV or PF sorting	PAPR
Entry into Equipment Corridor if less than 2 hours since TB, HIV, LCMV or PF sorting	N95
Disposal of TB, HIV, LCMV and/or PF waste in Equipment Corridor	N95
TB, HIV or LCMV sorting or †observation	PAPR
P. falciparum (PF) sorting or †observation	PAPR
P. yoelii and/or *P. berghei* sorting or †observation	N95
PAPR decontamination activities in Equipment Corridor	N95
Spill response	PAPR
*Facilities staff entries for repairs, routine maintenance, etc.	N95
*Inspections	N95

* Facilities and inspection entries must not take place within 2 hours after TB, HIV, LCMV or PF sorting, and not within 24 hours after TB/HIV/LCMV/PF culture sorting.

† Observation entry requires prior completion of respirator medical clearance and fit testing, and TB testing.

Figure 1. TB BSL-3 Floor Plan

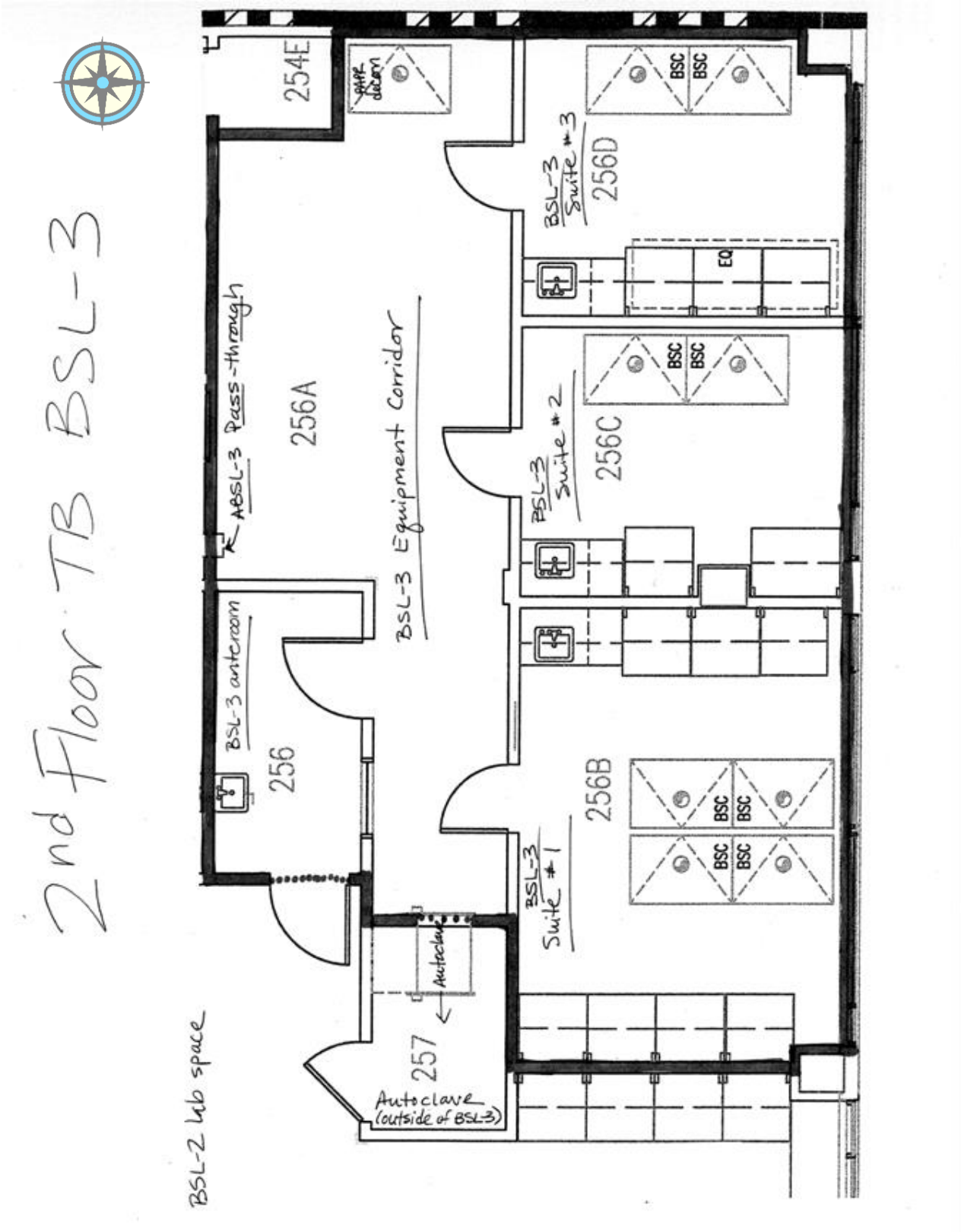

Figure 2. TB BSL-3 Training Documentation

TB BSL-3 Training Documentation

Personnel must complete this training before gaining access to the BSL-3

Name _____ **BSL-3 Trainer** _____

Position _____ **Principal Investigator** _____

Relevant Experience and Degree(s) _____

List types of experiments to be performed _____

***If applicable, approved for limited entry only when accompanied by:**

_____ ON _____

Comments: _____

Procedure for Gaining Access to the TB BSL-3

Contact EHS to obtain information about medical testing and/or respirator training.

Access Checklist:	Date
☐ Obtain Respiratory Medical Clearance (contact EHS)	___/___/___
☐ Provide TB Testing Policy Acknowledgement E-Signature *https://ws01.sbri.org/ESignatures/User/Create/48*	___/___/___
☐ Obtain TB Testing (contact EHS)	___/___/___
☐ Receive N95 Fit Test and Training from EHS	___/___/___
☐ Receive PAPR Training from EHS	___/___/___
☐ Review TB BSL-3 Manual and Complete Test *Manual - http://intranet.sbri.org/ehs/Pages/Safety_Manual/Safety%20Manuals.aspx Test - http://intranet.sbri.org/ehs/Pages/TB-BSL-3-Safety-Test.aspx*	___/___/___
☐ Meet with TB BSL-3 Director (David Sherman)	___/___/___
☐ In-lab training with TB BSL-3 Manager (R. Liao) *See training requirements below:*	___/___/___

TB BSL-3 In-Lab Training *(Contact TB BSL-3 Manager – R. Liao)*	Yes	N/A	Trainer's Initials	Supervisor's Initials
1. Entry/Exit (gown/degown)	_____	_____	_____	_____
2. Emergency Exit (fire/biohazard)	_____	_____	_____	_____

3. Emergency Procedures & Supplies	_____	_____	_____	_____
4. Waste Handling	_____	_____	_____	_____

TB BSL-3 Training Continued *(Contact TB BSL-3 Manager – R. Liao)*	**Yes**	**N/A**	**Trainer's Initials**	**Supervisor's Initials**
5. Autoclave	_____	_____	_____	_____
6. Supplies (stocking/ordering)	_____	_____	_____	_____
7. Disinfectant (use/refilling, reordering)	_____	_____	_____	_____
8. Decontamination/Spill Procedures	_____	_____	_____	_____
9. Centrifuges	_____	_____	_____	_____
10. Biological Safety Cabinet Operation	_____	_____	_____	_____
11. Incubators	_____	_____	_____	_____
12. Refrigerator/Freezer	_____	_____	_____	_____
13. BSL-3 Alarm System	_____	_____	_____	_____
14. Infected Culture Handling	_____	_____	_____	_____
16. Practical Respirator Training	_____	_____	_____	_____
17. Other _____	_____	_____	_____	_____

I acknowledge that I have studied and understood the Minimum BSL-3 Safety Procedures for Entry/Exit and the BSL-3 Procedures in the CENTER FOR INFECTIOUS DISEASE RESEARCH Safety Manual, Appendix N. I have received adequate training in these BSL-3 Procedures necessary to perform my objectives with safety, neatness, and technical expertise. I will request and receive additional training from my supervisor when I need to operate new equipment or work with new reagents or biologicals.

Trainee _____ Date _____

I verify that the trainee listed above has been trained in BSL-3 Safety Procedures and is prepared to work in the TB BSL-3 facility.

BSL-3 Trainer _____ Date _____

Principal Investigator _____ Date _____

BSL-3 Lab Director _____ Date _____

Biosafety Officer _____ Date _____

☐ Please return signed training documentation to EHS.

EHS Use only: ☐ Yes ☐ No All respirator and occupational health dates verified

___/___/___ Notification emailed to BSL-3 Lab Director

VI. References

http://www.lni.wa.gov/safety/rules/chapter/823
WAC 296-823 Bloodborne Pathogens. Washington State Department of Labor and Industries 2015.

Center for Disease Control and Prevention. http://www.cdc.gov/

Washington State Department of Labor and Industries. http://www.lni.wa.gov

Biosafety in Microbiological and Biomedical Laboratories, 5th Ed. - Centers for Disease Control and Prevention. US Department of Health and Human Services.
http://www.cdc.gov/biosafety/publications/bmbl5/

"Guidelines for Preventing the Transmission of Mycobacterium tuberculosis in Health-Care Settings, 2005," Morbidity and Mortality Weekly Report (MMWR) December 30, 2005 / 54(RR17);1-141.

ADDENDUM TO TB BSL-3 PROCEDURES – FLOW CORE TB-BSL-3

TB BSL-3 FLOW-CORE

The BSL-3 Flow Core facility, located in the Northwest corner of the 4th floor, sorts viable BSL-2 and BSL-3 agents. A map of the facility is appended. The aerosolization of these infectious agents presents an acute inhalation hazard to Flow Core personnel, and other staff members who have access to this facility such as the Facilities Department. To ensure understanding and safety compliance from Flow Core staff while working with all infectious agents, the BSL-3 Flow Core facility will adhere to the safety provisions and procedures outlined in the TB BSL-3 manual at all times. Following only the most conservative safety protocol will ensure that the necessary safeguards are in place to protect all personnel with Flow Core access.

A. Responsibilities

The Flow Core Manager is responsible for ensuring that all Flow-Core staff adhere to all provisions of this manual. Recommendations and updates to this appendix shall be provided by the Flow Core Manager as technologies and equipment are updated and new infectious agents are used. The Flow Core Scientific Advisor supervises the Flow Core Manager and reviews and approves all Flow Core projects, in conjunction with the IBC, prior to start of work. The TB BSL-3 Director, and IBC, reviews and approves all use of new infectious agents in the facility. The Flow Core Scientific Advisor and Flow Core Manager are responsible for determining and approving who may enter the BSL-3 Flow Core Facility. The TB BSL-3 Director must also approve all entry for TB-related work.

B. Infectious Agents Used in the Flow Core

BSL-2 and BSL-3 agents that are sorted in the BSL-3 Flow-Core facility must have a Flow Core-approved SOP, an IBC-approved Infectious Agent (IA) application, and an IBC-approved rDNA application (where required). The IA and rDNA applications for the grant work and infectious agent must include any planned use of the Flow-Core facility. If Flow-Core work needs to be added to a previously approved IA or rDNA application, an amendment must be submitted to the IBC, Flow Core Manager, and Scientific Advisor, for review and approval. This policy also applies to collaborators, visiting equipment users, and other external users.

C. Visitor Policy

The only personnel allowed to enter the BSL-3 Flow-Core facility are those that have completed both the occupational health requirements and the Center for Infectious Disease Research TB BSL-3 training program. Entry by such individuals must be approved, in advance, by the BSL-3 Director, Flow Core Manager, and Scientific Advisor. Under no circumstances shall unauthorized personnel or visitors enter the BSL-3 Flow Core Facility. Individuals who want to observe a sort can do so remotely by using Remote Desktop and Communicator for communication with Flow-Core personnel by telephone. Contact the IT department for further details on how to set up the shared desktop capabilities.

D. Minimum PPE Requirements

All PPE requirements, donning and doffing procedures, and spill response protocols outlined in the TB BSL-3 Safety Manual must be followed for BSL-3 Flow-Core entry and exit. In addition to these requirements, Flow-Core personnel are required to wear a PAPR for entry within 3 hours of a sort. All other entry into the facility, including into the equipment corridor, required an N95 to be worn as

minimum respiratory protection. Other personnel that require intermittent access to this facility, such as EHS and Facilities, are not allowed to enter during or within 3 hours of a sort.

E. Transport of Infectious Materials into the Flow-Core

The Flow-Core BSL-3 facility is in a separate location from the other HIV and TB BSL-3 laboratories. To transport infectious materials from the other BSL-3 environments to the Flow-Core through the general laboratory space, the following decontamination procedure must be followed:

1. Cultures must be contained in a primary water-tight container that is sealed with either internal threads or an O-ring.
2. The primary container must be wiped down with an approved disinfectant for the infectious agent.
3. The primary container must be placed within a secondary container (i.e. ziplock bag) with absorbent material (i.e. paper towels).
4. The outside of the container must be decontaminated with an approved disinfectant for the biosafety level of the area from which the culture is being prepared.
5. The outside of any additional containers needed for transporting the prepared primary and secondary containers on ice must also be decontaminated before being brought into the Flow-Core.
6. All containers used for transport must be clearly labeled with contents, infectious agent and date.

This policy also applies to infectious agents being brought into the Flow-Core from a BSL-2 environment.

F. Removal of Material from the Flow-Core

After the completion of a Flow-Core SOP, any FACS tubes or other containers containing either BSL-2 or BSL-3 agents must be prepared as follows before being transported out of the Flow-Core BSL-3 environment:

1. FACS tubes or other containers must be closed and wiped down with an approved mycobacterium disinfectant (See TB BSL-3 manual or ask the Flow-Core facility manager).
2. Place the decontaminated sample tubes into a secondary container (i.e. zip lock bag) with an absorbent material and wipe the outside down with an approved mycobacterium disinfectant.
3. Place the prepared sample package in a Nalgene Bio Transport Carrier and wipe off the outside with an approved mycobacterium disinfectant.

   NOTE: Live BSL-3 agents may not be transported or removed from the facility unless all decontamination procedures are followed, and the intended location is another BSL-3 facility.

G. Waste Disposal Procedures

Procedures for decontaminating liquid and solid biohazardous wastes and general decontamination of PPE must be followed as described in the TB BSL-3 manual, or as determined for an infectious agent if TB-BSL-3 decontamination procedures are insufficient for deactivation.

H. Decontamination Procedures

Decontamination of the flow cytometry machine should be performed after every sort following the most current SOP that has been approved by the Flow-Core Manager, Biosafety Officer, EHS Manager, and Scientific Advisor. This procedure will be demonstrated to each new staff member during the training period and will be periodically monitored by the Flow-Core Manager to ensure each individual is properly disinfecting the machine.

Figure 3. TB BSL-3 Flow Core Floor Plan

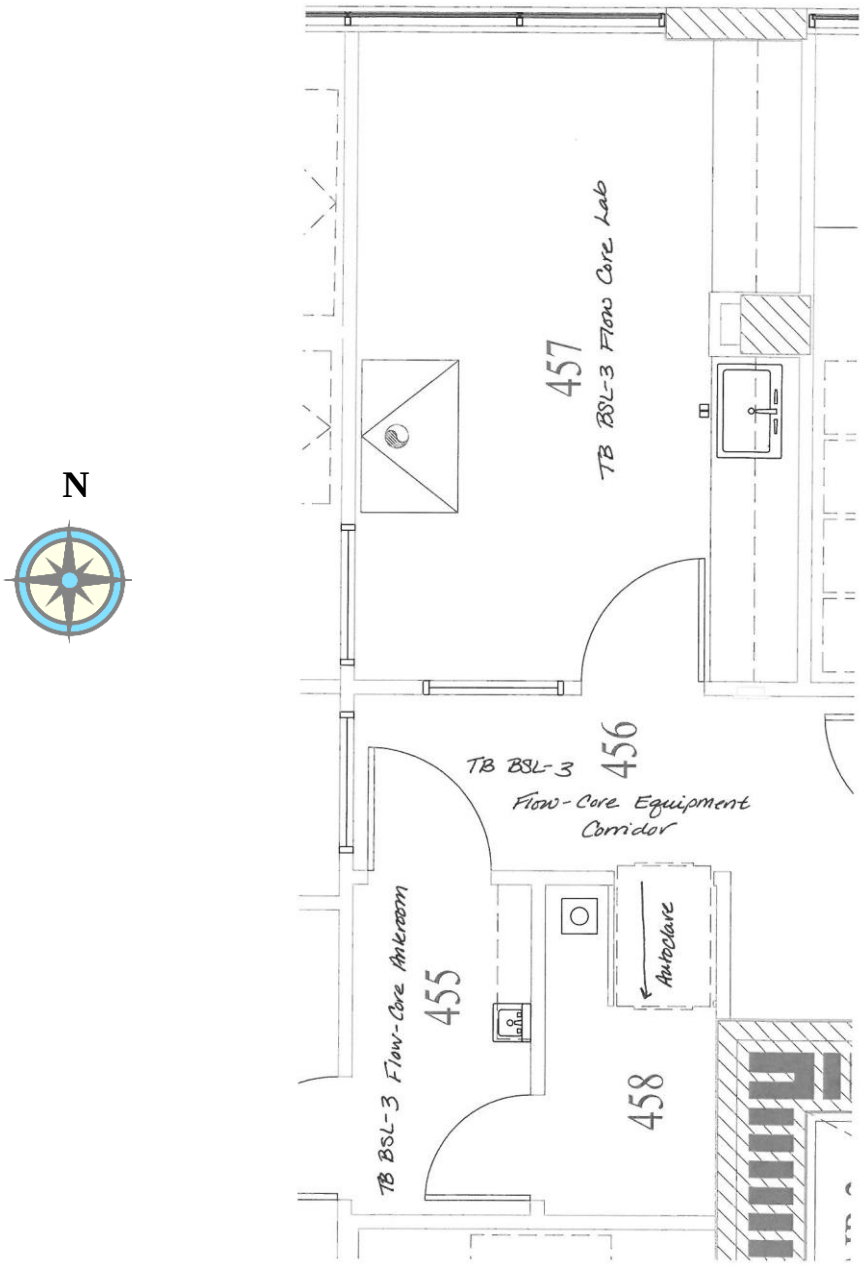

Figure 4. TB BSL-3 Flow Core Training Documentation

Flow Core BSL-3 Training Documentation

Personnel must complete this training before gaining access to the Flow Core (FC) BSL-3

Name _____ **BSL-3 Trainer** _____

Position _____ **Principal Investigator** _____

Relevant Experience and Degree(s) _____

List types of experiments to be performed _____

***If applicable, approved for limited entry only when accompanied by:**

_____ ON _____

Comments: _____

Procedure for Gaining Access to the TB BSL-3

Contact EHS to obtain information about medical testing and/or respirator training.

Access Checklist:	**Date**
☐ Obtain Respiratory Medical Clearance (contact EHS)	___/___/___
☐ Sign TB Testing Policy Acknowledgement *https://ws01.sbri.org/ESignatures/User/Create/48*	___/___/___
☐ Obtain TB Testing (contact EHS)	___/___/___
☐ Receive N95 Fit Test and Training from EHS	___/___/___
☐ Receive PAPR Training from EHS	___/___/___
☐ Review TB BSL-3 Manual and Complete Online Test	___/___/___

Manual:http://intranet.sbri.org/ehs/Pages/Safety_Manual/Safety%20Manuals.aspx
Test: http://intranet.sbri.org/ehs/Quizzes/FC-BSL-3-Safety-Test.aspx

☐ Meet with Flow Core BSL-3 Director (Kevin Urdahl)			___/___/___	
☐ In-lab training with FC BSL-3 Manager (W. DeBusk) *See training requirements below:*			___/___/___	
FC BSL-3 In-Lab Training *(Contact FC BSL-3 Manager – W. DeBusk)*	**Yes**	**N/A**	**Trainer's Initials**	**Supervisor's Initials**
1. Entry/Exit (gown/degown)	_____	_____	_____	_____
2. Emergency Exit (fire/biohazard)	_____	_____	_____	_____
3. Emergency Procedures & Supplies	_____	_____	_____	_____
4. Waste Handling	_____	_____	_____	_____

TB BSL-3 Training Continued *(Contact FC BSL-3 Manager – W. DeBusk)*	**Yes**	**N/A**	**Trainer's Initials**	**Supervisor's Initials**
5. Autoclave	_____	_____	_____	_____
6. Supplies (stocking/ordering)	_____	_____	_____	_____
7. Disinfectant (use/refilling, reordering)	_____	_____	_____	_____
8. Decontamination/Spill Procedures	_____	_____	_____	_____
9. Centrifuges	_____	_____	_____	_____
10. Biological Safety Cabinet Operation	_____	_____	_____	_____
11. Incubators	_____	_____	_____	_____
12. Refrigerator/Freezer	_____	_____	_____	_____
13. BSL-3 Alarm System	_____	_____	_____	_____
14. Infected Culture Handling	_____	_____	_____	_____
16. Practical Respirator Training	_____	_____	_____	_____
17. Other _____	_____	_____	_____	_____

I acknowledge that I have studied and understood the Minimum BSL-3 Safety Procedures for Entry/Exit and the BSL-3 Procedures in the CENTER FOR INFECTIOUS DISEASE RESEARCH Safety Manual, Appendix N. I have received adequate training in these BSL-3 Procedures necessary to perform my objectives with safety, neatness, and technical expertise. I will request and receive additional training from my supervisor when I need to operate new equipment or work with new reagents or biologicals.

Trainee _____ Date _____

I verify that the trainee listed above has been trained in BSL-3 Safety Procedures and is prepared to work in the TB BSL-3 facility.

BSL-3 Trainer _____ Date _____

Principal Investigator _____ Date _____

BSL-3 Lab Director _____ Date _____

Biosafety Officer _____ Date _____

☐ Please return signed training documentation to the EHS.

_____

EHS Use only:

☐ Yes ☐ No All respirator and occupational health dates verified

___/___/___ Notification emailed to BSL-3 Lab Director

ADDENDUM TO TB BSL-3 PROCEDURES – HIV/TB CO-INFECTION STUDIES

A. Requirements for Performing HIV Co-Infection Work in the BSL-3

HIV/TB coinfection experiments may require bringing blood or purified cells from HIV-infected donors into the TB-BSL-3. Samples will be aliquotted to individual FACS tubes before M tuberculosis is added. Samples will be incubated with M tuberculosis for up to 96 hours. Following incubation, samples may be processed for flow cytometry, fluorescence, colony counts, microscopy, or RNA analysis. All personnel working with HIV/TB shall be required to follow all requirements of the IBC-approved rDNA and Infectious Agent applications for such work. Training and HIV testing offers shall be completed as specified in the approved applications. Personnel performing this work must be fully trained to work with HIV and M. tuberculosis, and must also follow all established BSL-3 procedures. All staff working in the vicinity shall be notified of the use of this agent via written notification, requirement to review this document, educational sessions (as required), and applicable signage provided by lab staff when HIV/TB coinfection work is in process.

Use of HIV/M. tuberculosis is to be performed in a hood, incubator and centrifuge that has been designated for HIV/TB coinfection work. Prior to and after performing this work the hood, incubator and centrifuge that have been used must be cleaned using standard TB-BSL-3 disinfecting agents. When common equipment is in use, signage must be posted. In addition, full standard M. tuberculosis personal protective equipment required for work in the BSL-3 must be worn while performing the HIV/TB coinfection work. No sharps will be used for this work.

B. Waste Disposal Requirements

All HIV/M. tuberculosis-contaminated waste shall be disposed of using the same procedures as required for TB-BSL-3 waste. All biohazard waste bags containing HIV/M. tuberculosis must be clearly labeled as such. Waste shall be bagged only by those trained to perform this work.

C. Handling Emergencies

Spills of HIV/M tuberculosis material shall be handled in accordance with standard TB-BSL-3 procedures, as HIV is easily neutralized by all M. tuberculosis disinfecting agents. Other individuals utilizing the laboratory must be immediately notified of any spills, in addition to the Principal Investigator, Biosafety Officer, BSL-3 Laboratory Director, and EHS Manager.

Occupational exposures must be reported immediately to the Principal Investigator, BSL-3 Laboratory Director, Biosafety Officer, and EHS Manager. Treatment and care for such exposures are outlined in the approved Infectious Agent applications for this work. The Center strongly advises that exposed individuals seek treatment immediately. EHS shall ensure that an emergency exposure response envelope specific to HIV/TB coinfection work is posted in the BSL-3 laboratory.

D. Transport of HIV-Infected Blood into the BSL-3

When HIV-infected blood or other HIV-containing biological materials are brought into the BSL-3 for processing, the tubes or flasks must be packaged, transported, unpacked and handled in the biosafety cabinet using standard BSL-3 procedures. These containers should be cleanable or disposable by means of autoclave or approved Tuberculocidal agent. An additional wipe down of containers with 70% ethanol is recommended prior to transport into the BSL-3.

E. Transport of HIV/*M. tuberculosis* Materials Out of the BSL-3

If HIV/M. tuberculosis-containing cultures need to be removed from the BSL-3 (for example, if they are to be analyzed by flow cytometry) the infected cells must be fixed with 2% paraformaldehyde for one hour prior to removal to inactivate both the HIV and M. tuberculosis. Tubes or plates containing fixed cells must be individually sprayed with approved Tuberculocidal agents (1% T.B.Q. or Accel TB / PREEmpt) prior to removal from the Biosafety cabinet, and placed into a plastic, cleanable or autoclavable, secondary container, in accordance with transport of M. tuberculosis requirements. The secondary container itself must also be sprayed with the tuberculocidal agent prior to removal from the BSL-3 facility.

ADDENDUM TO TB BSL-3 PROCEDURES – BSL-3 REQUIREMENTS FROM THE BMBL

The following is a direct excerpt from the Centers for Disease Control (CDC) Publication: *Biosafety in Microbiological and Biomedical Laboratories (5th Edition).* Center for Infectious Disease Research follows all applicable standards outlined in the document. Specific examples are called out in the TB BSL-3 Manual and supporting documentation.

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices. A BSL-3 laboratory has special engineering and design features. The following standard and special safety practices, equipment, and facility requirements apply to BSL-3.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
    a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
    c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
    d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:

   a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
   b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
   a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
   b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective

equipment and other containment devices, such as a centrifuge safety cup or sealed rotor must be used.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
2. Workers in the laboratory where protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.
3. Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers:
    a. Changes gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
    b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted. Access to the laboratory is through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.
    a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
    b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
    c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.
    d. Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
   a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
   b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. All windows in the laboratory must be sealed.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available in the laboratory.
9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from "clean" areas toward "potentially contaminated" areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
   a. Laboratory personnel must be able to verify directional airflow. A visual monitoring device, which confirms directional airflow, must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.
   b. The laboratory exhaust air must not re-circulate to any other area of the building.
   c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.
10. HEPA filter housings should have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.
11. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.
12. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).
13. Equipment that may produce infectious aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
14. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
15. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices, such as biometrics.
16. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.