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Introduction to the Hildebrand Biosafety Level 3 (BSL3) Facility

This manual describes the general procedures for working with Biosafety Level 3 pathogens (specifically *M. tuberculosis*) in the Hildebrand BSL3 facility. Accompanying this manual is a separate document describing all approved protocols used in this BSL3 facility.

Working with BSL3 pathogens requires diligence from the laboratory worker to maintain safe laboratory conditions. This includes extensive knowledge of both the pathogen and the procedures, proper training and certification, and rigorous adherence to safety practices. Failure to meet any of these expectations will result in removal of BSL3 access privileges.

*Mycobacterium tuberculosis* is the major pathogen used in the Hildebrand BSL3 Facility and every user must be knowledgeable of the specific risks and precautions associated with *M. tuberculosis* work. Any pathogen other than *M. tuberculosis* used in the Facility must first be approved by the UC Berkeley Committee on Laboratory and Environmental Biosafety (CLEB), the EH&S Biosafety Officer (BSO), and the internal Biosafety Committee (iBSC). Additional training will be involved for BSL3 pathogens other than *M. tuberculosis*.

The user is responsible for being well versed in the experimental design and execution of each protocol to be done in the BSL3. Also, all procedures must be approved and added to the Approved Protocols manual prior to being used in the BSL3 facility.

Training of users will take place prior to any work in the BSL3. Each user is required to take biosafety training through EH&S as well as BSL3-specific training. After the user has displayed adequate competency in safely handling BSL3 pathogens, the user will be tested under mock BSL3 conditions and evaluated for knowledge of procedures and technique. A short evaluation period is also required before the user can work in the BSL3 unsupervised.

Finally, adherence to safety practices is paramount to all BSL3 work. An unsafe worker jeopardizes the health and safety of all workers in the BSL3. Any reports of unsafe working conditions will be critically evaluated and appropriate measures will be taken, including retraining/certification, reduced privileges, or banning from the facility.
# Contact Information

## Working Hours:

<table>
<thead>
<tr>
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<th>Role</th>
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## Healthcare

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## Emergency Contacts:

<table>
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<tr>
<th>Role</th>
<th>Name</th>
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<tbody>
<tr>
<td>Emergency Response Hotline</td>
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<tr>
<td>Emergency Response</td>
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<td>911</td>
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Requirements for beginning work in the BSL3 Facility

In order to work in the BSL3 facility, all potential users must complete the following requirements.

- Enroll in the medical surveillance program
- Be fitted for a respirator
- Complete EH&S general biosafety training course
- Complete the BSL3 safety training
- Demonstrate BSL3 safety techniques
- Be evaluated by a BSL3 user during a short trial period

1. Medical surveillance

The first step towards working in the BSL3 facility is to enroll in the TB surveillance program. All personnel working in the BSL3 must be tested regularly for exposure to M. tuberculosis. TB surveillance consists of a Tuberculin skin test (PPD test) or chest X-ray. Both of these tests are administered by the Tang Occupational Health Clinic (www.uhs.berkeley.edu).

- Contact the Facility Director to add you to the list of personnel needing TB surveillance
- Prepare an IOC form to pay for the test (check price with Clinic Nurse)
- Bring a copy of any prior PPD tests or chest X-rays
- If you have not been PPD tested – you must obtain a 2 step test (get tested twice)
- If you are PPD+ (including those vaccinated with BCG) you are required to have a basal chest X-ray unless you have record of one from the last 12 months
- Give the result of either the PPD test or chest X-ray to the Facility Director

All personnel working in the BSL3 are required to be tested every 6 months. The Facility Director will keep track of TB surveillance scheduling and contact personnel when they are due for their tests. However, it is the responsibility of the user to go to the Tang Occupational Health Clinic, have the test administered and deliver the results to the Facility Director. Failure to do this will result in barring the user from the BSL3 facility until the tests are completed.

TB surveillance is required every 6 months:

- PPD- individuals are required to have a PPD skin test
In the event of conversion to PPD+, the user must immediately contact the Facility Director, who will then notify the Biosafety Officer and the Principal Investigator.

- PPD+ individuals must complete a semi-annual health survey.
- All symptoms of illness that may be related to infectious organisms being used in the lab must be brought to the attention of the Facility Director, who will then notify the Biosafety Officer and the Principal Investigator.

PPD screening consists of subcutaneous injection of tuberculin, which must be read 48-72 hours later.

PPD screening times:

- Monday, Wednesday, Friday ............................................. 9 to 10 AM
- Tuesday ................................................................. 4 to 5 PM
- Thursday (PPD reading and symptom reviews only) ............... 4 to 5 PM

2. Respirator fit testing

Respirators must be worn at all times in the BSL3 facility. Proper fit of the respirator is critical for function and must be tested by EH&S.

- Contact Sara Souza (sarasouza@berkeley.edu) for respirator fit testing
- Complete the respirator medical clearance form prior to being tested and submit to Tang Center for review

All users must be fit tested every year and are responsible for staying up to date. Failure to complete the required fit testing will result in the user being barred from entering the BSL3 facility.

3. EH&S biosafety training

UC Berkeley EH&S (www.ehs.berkeley.edu) requires two training classes for work with pathogens in the BSL3 facility.

- Complete the general biosafety class - Biosafety for human, animal and plant pathogens. Enroll for this class at hrweb.berkeley.edu/ice/home (select “biosafety for human, animal and plant pathogens” Course Code “CBIO”). The course is 2 hours long and is offered approximately once per month.
4. BSL3 safety training

The BSL3 safety training is the most intensive and specific training required to work in the BSL3 facility. All aspects of the BSL3 safety training are outlined in this manual. The applicant is expected to have thoroughly read this manual before training begins.

To begin BSL3 training, an active BSL3 user will be chosen by the Facility Director to introduce the applicant to generalized BSL3 procedures and provide specific technical training based on the submitted protocols. All training must include identification and control of the hazards with which personnel will be working. Furthermore, regardless of the techniques required for the proposed protocol, the training program must include performing mock procedures that cover the following topics:

- Adherence to general lab safety procedures, such as wearing double gloves and sterilizing gloved hands before removing them from the biosafety cabinet.
- Setting up, cleaning out, and properly using the biosafety cabinets.
- Bringing materials into and out of the cabinet.
- Culturing and manipulating *M. tuberculosis* safely, with emphasis on the importance of avoiding aerosol generation during all operations.
- Performing the essential procedures required of most protocols, such as centrifugation, plating, and incubating.
- Disposing of waste.
- Bringing materials out of the BSL3
- Following emergency procedures, especially what to do in the event of a spill. Particular emphasis must be placed on the reporting of spills and accidents, no matter how minor the incident may seem.

At least two training sessions must be performed, with additional sessions at the discretion of the either the trainer or applicant. The applicant will be responsible for completing the Documentation of Training Form.

5. BSL3 safety testing

Following the training period, the applicant must demonstrate to the Facility Director proper BSL3 safety practices. The test will require the applicant to demonstrate their ability to follow all the approved biosafety practices outlined in this manual. Typically, the applicant will have to complete a mock procedure that covers general *M. tuberculosis* culturing and manipulation. If the applicant fails to show competence in properly handling *M. tuberculosis*, more training will be required specifically focusing on problems that arise during testing and the
applicant will have to be tested again by the Facility Director.

After satisfactorily demonstrating biosafety techniques, the Facility Director will approve the applicant to work with live bacteria.

6. **Supervised trial period**

Given the complexities and risks associated with BSL3 work, any new user must undergo a supervised trial period. For the first 2 sessions of work in the BSL3, an existing BSL3 user must be present to help the new user if needed and observed the user’s technique. The new user will be responsible for documenting the date, time and who supervised these initial sessions and giving this information to the Facility Director.

After the supervised trial period, the new user will confer with the Facility Director to discuss any additional problems or suggestions and be able to work independently in the BSL3.

**Personnel not working directly with M. tuberculosis**

Physical Plant personnel, vendors and repair person, visitors, and inspectors who need to access the BSL3 lab for short periods of time must sign the **Acknowledgement of Risk and Agreement to Follow Guideline** form prior to entering the facility. Personnel will be given brief training by the Facility Director and Biosafety Officer or qualified designate on the rules of the facility and correct use of personal protective equipment. Personnel must contact the Biosafety Officer and Facility Director prior to entry and receive clearance.

Personnel wishing to use the BSL3 facility to work on pathogens other than M. tuberculosis must first get approval from CLEB, EH&S, the Facility Director, and the PI. Such personnel are required to fully follow the above guidelines for beginning work in the BSL3. Procedural testing will focus on a protocol that is appropriate for the work being done and will be done by the Facility Director.
Description of *Mycobacterium tuberculosis*

1. **Background**
Tuberculosis has existed for centuries, and used to be called “consumption”. It is a serious disease that usually attacks the lungs, but can also affect other parts of the body, including the brain, the lymph nodes, and bones. Although most TB infections are curable with antibiotics, it continues to be a major health problem and kills as many as three million people worldwide every year. However, multiple-drug resistant TB is increasing world-wide, complicating treatment.

2. **How TB is Spread**
*Mycobacterium tuberculosis* is an airborne pathogen. It is usually spread through the air from person to person, when someone with infectious TB disease coughs or sneezes. In a laboratory setting, mechanical aerosol generation can also lead the release of particles which can cause infection if inhaled. Likewise, direct inoculation via injection into the bloodstream can also cause infection.

3. **Tuberculosis Infection and TB Disease**
Most people who are exposed to TB bacteria do not develop TB disease. In some cases, the person’s immune system is able to kill the TB bacteria. When this doesn't happen, the bacteria can remain alive but inactive in the body, and this is called TB infection. A person with TB infection has no symptoms, is not sick, and poses no risk of spreading the bacteria.
TB infection may develop into TB disease if the infected person's immune system cannot stop the TB bacteria from growing. The risk of developing TB disease is highest in the first two years after someone is infected. About 10% of infected adults will develop TB disease at some point in their lives.

4. **Risk Factors for Developing TB Disease**
Anyone with a weakened immune system is at greater risk of developing TB disease. Conditions that weaken the immune system include HIV infection, end stage kidney disease, diabetes, cancer, organ transplants, silicosis, abuse of alcohol or drugs, and low body weight.
Babies, pre-school children, and the elderly are also at greater risk because their immune systems are weaker than those of healthy adults.
Also, certain population groups have an increased risk of TB infection. These groups include people born in or traveling to countries where TB is widespread, homeless people, and people who live in long-term care or correctional facilities. People who work with any of these groups (such as health care workers) are also at greater risk.
5. The Symptoms of TB Disease

The symptoms of TB disease in the lungs can include a bad cough that lasts longer than two weeks, pain in the chest, coughing up blood or phlegm, weakness or feeling very tired, weight loss, lack of appetite, chills, fever, and night sweats.

A PPD skin test can determine whether you have TB infection. If you do, it is important to protect your immune system from becoming weak. Treating TB infection with antibiotics will help your immune system fight the TB bacteria and prevent the development of TB disease.

The BCG vaccine is still administered to many children around the world (not in the US), despite its dubious effectiveness for preventing TB in adults. BCG vaccination leads to reactivity against PPD and thus can lead to false-positive skin tests. Persons known to be vaccinated with BCG should not take the skin test and should be given a chest X-ray to rule out active disease.

You may also need additional tests or chest x-rays to determine whether you have TB disease. Anyone with TB disease must take antibiotics for at least six months to kill all of the TB bacteria.

People who do not finish a full course of antibiotic treatment are at greater risk for developing a strain of TB that is drug resistant and harder to cure. They also pose a greater risk of spreading TB to others.
Entering and exiting the BSL3 facility

1. Using personal protective equipment (PPE)

Designated personal protective equipment (PPE) must be worn by every person entering the BSL3. All PPE will be provided and will be stocked in either the ante-room or entry vestibule. Change into all PPE in the ante-room before entering the BSL3 facility. Required PPE includes:

- Respirator (N95 or PAPR)
- Eye protection (goggles or PAPR)
- Double gloves (thick inner pair, standard outer pair – preferably latex)
- Shoe covers
- Disposable, back closure lab gown

Put on Shoe covers first, then put on and tie the gown in the back. Place inner gloves over the cuffs of the gown and tape them to the gown to prevent gaps. Put on the second pair of gloves over the first. Form respirator to the face and put on eye protection.

All personnel must be certified physically able to wear a respirator and must be fit-tested by EH&S to ensure proper fit. One must be retested annually or if significant changes to the fit area occur, such as a sizable weight change or allowing growth of any facial hair. It is the job of all BSL3 users to detect compliance failures and report them to the Facility Director. Anyone not in compliance with the surveillance plan will be required to be retested.

PAPR devices will be available as an extra safeguard for performing potentially aerosol-generating procedures.

2. Entering the BSL3 barrier

Before opening the door from the Ante-room to the BSL3, complete the following checklist:

- Be sure you are wearing proper PPE
- Note - Name, Date, Time, and Laboratory affiliation in log book
- Confirm negative pressure in the BSL3
  - Proper air balance is indicated by a green light on the meter next to the door
If there is a problem with the air balance, i.e. if the indicator light is either red or yellow, do not open the door. Call the Facility Director immediately and do not let others enter the laboratory.

• Only open the inner door to the BSL3 facility if the outer door of the ante-room is closed.

3. Exiting the BSL3 facility

Before exiting the BSL3 facility:

• Remove the outer layer of gloves and place in biohazard waste container inside the BSL3
• Look through the glass into the ante-room to be sure the door from the ante-room to outside is not open
• Enter the ante-room
• Remove shoe coverings and place in the biohazard container
• Remove and dispose of the gown, masks and latex gloves in the biohazard container
• Rinse off protective eye ware and return to shelf
• If using a PAPR, disconnect the hood and plug in the HEPA unit
• Note time of departure on the sign in sheet
• Wash hands thoroughly with antibacterial soap

Running the autoclave

All trash must be autoclaved before it can be removed from the BSL3 facility. All PPE must also be autoclaved before being picked up by EH&S. Each user is responsible for autoclaving trash and must be familiar with autoclave function.

The autoclave can only be set from the “clean” side – in the autoclave room. Once the autoclave is properly set, the cycle can be started from the BSL3 side remotely.

Always use an autoclave tray. Report any problems immediately to the Facility Director.

1. Autoclaving trash

• Check that the Jacket is on (to turn on - press the Jacket button)
  o Jacket pressure should read 22 psi
• Trash should be run in the “Dry” cycle
  o Press – 1 (cycle selection)
  o Press – 2 (Dry)
• Check the cycle you are running
  o Temperature = 121ºC (250ºF)
  o Time = 30 minutes
• Press – 9 (exit) until you reach the main menu (3x)
• Press the Run button
• Press Enter 2 times to begin cycle

If the cycle is set correctly, the autoclave can be started by pressing the Remote start button on the BSL3 side of the autoclave twice.

2. Autoclaving plates

Plates are the only trash that should contain live *M. tuberculosis* and requires a longer cycle. Follow the above directions except:

• **Set cycle time = 60 minutes**

Bags of plates must be completely contained within a tray to prevent molten agar from blocking drain.

3. Autoclave problems

Before removing autoclaved trash for pickup by EH&S, check the screen to be sure that the cycle completed successfully.

**If the cycle did not complete do not open autoclave!**

Reset the autoclave and run the cycle again following the above instructions. If the autoclave fails, notify the Facility Director immediately. Post signs on the autoclave instructing users not to open the autoclave.
Working with *M. tuberculosis* in the Biosafety Cabinet (BSC)

Direct manipulation of *M. tuberculosis* is only allowed inside a prepared Biosafety Cabinet (BSC). All *M. tuberculosis* cultures outside of the BSC must be in secondary containment unless specified otherwise.

1. Preparing the BSC

Once *M. tuberculosis* is introduced into the BSC, all equipment, materials and hands must be disinfected prior to removal from the BSC. Therefore, proper preparation of the BSC is essential to safely handling *M. tuberculosis*.

- Check the airflow indicator on the front panel of the BSC - negative pressure indicates that the unit is working properly and is safe to use
- Place a red biohazard bag in polypropylene container
- Bring in an open container of disinfectant (Vesphene Ilse diluted 1:40 with water) with a soaked disposable blue towel
- Place materials and equipment that will be used including pipettors, pipets, pipet tips, sterile culture flasks, centrifugation tubes, media, etc., inside the BSC

Prior to the introduction of *M. tuberculosis*, materials may be taken in and out of the BSC without further precaution.

2. Working with *M. tuberculosis* in the BSC

Once sealed containers of *M. tuberculosis* have been placed inside the BSC nothing is to be removed without being wiped with disinfectant. Hands must be wiped thoroughly with disinfectant and removed from the BSC immediately. The outer layer of gloves should be discarded whenever there is the possibility of contamination with *M. tuberculosis*. All disposable materials that come into contact with *M. tuberculosis* are sterilized with Vesphene before being placed into the biohazard bag. Liquid waste is to be disinfected by addition of equal volumes of Vesphene in tightly sealed containers and placed into the biohazard bag.

3. Disinfection and removal of objects from the BSC

Objects are not to be removed from the BSC if there are open containers of *M. tuberculosis*. Objects to be removed will be wiped thoroughly with Vesphene and
can be carried directly out, if gloves have been wiped with Vesphene. Alternatively, an area at the edge of the cabinet can be wiped with Vesphene, and materials to be removed from the BSC may be wiped thoroughly and placed on this sterilized area and then removed immediately. Vesphene is the only allowable disinfectant; ethanol is never used except to remove the residue from Vesphene. Reagents and materials that are to be reused will be clearly labeled with users initials and “open only in BSC” then tightly sealed, outer surfaces disinfected, and removed as above.

4. Disinfection of the BSC

At the end of a procedure, all objects should be removed from the BSC and the entire BSC should be disinfected so it will be clean for the next user.

- Remove objects from the BSC as described above
- Seal the biohazard trash bag with a rubber band
- Wipe all exposed surfaces of the bag with disinfectant
- Removed sealed trash bag from the BSC and place in large biohazard bag adjacent to BSC
- Sterilize the cabinet with disinfectant in a “wave of cleaning”
  - All objects that are to remain in the BSC are placed on one side, the other side of the cabinet is sterilized by wiping all surfaces with a Vesphene-soaked cloth. Equipment is then wiped and placed on the sterile side and the “wave” continues to the other side.
- Remove the container of Vesphene
- Clean the BSC with a solution of 70% ethanol to remove the residue of Vesphene
- Turn off the light - leave the blower ON (for long BSC shutdown - close sash, turn off blower and turn on UV light)

Clean up the area around the BSC and the benches and place any trash in the large Biohazard bins. Turn off all equipment and inspect the BSL3 for hazards.

General techniques

- General microbiological aseptic technique is required in the BSC. If tube caps are placed on the surface of the BSC, it should be cap-up. Disposable loops or spreaders are to be used.
• Stable test tube racks and plastic boxes for culture flasks will be used to prevent a spill inside the BSC. All tubes and flasks will be properly sealed prior to removal from the BSC.

• Nothing may be removed from the BSC without every surface wiped with disinfectant.

• After use, pipettes will be rinsed (up and down pipetting once) with disinfectant, and discarded in the trash bag in the BSC.

• All cultures and surfaces of trash must be disinfected with Vesphene prior to placement into the trash bag. The only exception to this rule is agar plate disposal (see below).

• User will limit the number of items in the BSC during use, since clutter interferes with the airflow in the BSC.

1. Handling of liquid cultures

Special care must be taken when handling liquid cultures to avoid spilling of any liquid inside or outside the BSC. Also avoid generating aerosols, even inside the BSC. All manipulations of cultures must be done in the BSC.

• Supernatants will be poured or pipetted into a deep container with disinfectant.

• All pipetting is done slowly with a pipette-aid and care is taken to minimize expulsion of air into cultures.

• Liquid culture is grown in 3 types of containers.
  o Inkwell bottles or screw-cap centrifuge tubes are used to grow up small colonies into larger liquid medium. Inkwell bottles or centrifuge tubes containing growing cultures are placed within a large Tupperware container in the incubator.
  o Larger roller bottles are used to grow up frozen stocks of liquid culture. Only thick-walled Corning “vacuum filter” roller bottles may be used. Roller bottles are kept in the roller bottle incubator. Before opening the roller bottle incubator, users should check the interior of the incubator for cracked bottles and leaks.
  o Tissue-culture flasks with plug seal caps can also be used for standing cultures. If necessary, flasks can be capped with plug seal caps containing a 0.2 µm filter to allow gas exchange. Take special precaution to prevent any liquid from getting on these caps.
• Special care should be used when carrying roller bottles and tissue culture flasks from the incubator to the BSC.
  o If simply moving from the incubator to the adjacent BSC, carefully move only one culture at a time holding with both hands.
  o If you need to move cultures somewhere else in the BSL3, then a secondary container (such as an autoclave tray) should be used to house the roller bottles or flasks in transit.

2. Plating and handling of plates

All plating takes place in the BSC. Plates never leave the BSC without being in secondary containment. Plates can be wrapped in parafilm and a second layer aluminum foil or be placed in specialized canisters.

To photograph plates:

• Seal each plate with parafilm
• Wipe each plate with Vespheone and place in a clean container
• Wipe out the container and carry to camera station
• Photograph each plate individually and leave the rest inside the secondary container
• Wipe the camera station with Vespheone
• Return plates to the BSC

Plate disposal is the only exception to the “no live TB in the trash” rule. The reason for this is that adding Vespheone to agar plates is extremely messy (which causes a hazard) and TB colonies are so clumpy that penetration of a chemical disinfectant is likely to be incomplete.

• Plates containing TB are put inside small biohazard bag inside the BSC
• The bag is sealed and wiped thoroughly with Vespheone
• Place this bag inside another bag, rubber banded shut, and wipe out of the BSC
• The double-bagged plates are autoclaved immediately
• If the autoclave is busy, then the bag is left inside an operating BSC until it can be sterilized

3. Pipetting

Pipetting can generate aerosols. Only pipettes and pipet tips with aerosol barriers are allowed in the BSL3. Aerosols are minimized by avoiding blowing
out the contents of a pipette completely and by preventing the formation of bubbles. To avoid aerosol generation, whenever pipetting TB into a container, pipet onto the side wall of the container, and be careful not to eject any air after all of the liquid has been ejected. Similarly, avoid accidental intake of air when drawing liquid into a pipet or pipet tip. After pipetting, clean the inside and outside of the pipet by drawing Vesphene into the pipet and then allowing it to drain back into the pot of Vesphene. After the pipet has been rinsed, it can be disposed of in the waste bag.

For micropipet tips, following ejection of liquid, do not release the pipetman piston allowing air to be drawn into the pipet tip. Instead, holding the pipetman depressed, immerse the pipet tip in Vesphene and draw Vesphene into the tip. The tip can then be disposed of into the waste bag.

4. **Freezing Cultures**

TB cultures can be frozen in their 7H9 growth media.

- Prepare 2ml cryovial tubes by labeling them on both the side and cap of each tube and then place them into a rack
- Pipet 1-2ml of culture into each tube
- Deliver liquid along the side of the tube to avoid splashing and avoid producing bubbles
- Tighten the caps on all of the tubes
- Completely wash off an empty rack with Vesphene in the BSC
- Individually wipe down each cryovial and place them into the clean, empty rack
- Remove the entire rack from the BSC
- Outside the cabinet, transfer each tube into a clearly labeled freezer box and place the box into a metal tower in the freezer

5. **Heat killing *M. tuberculosis***

Heat killing of *M. tuberculosis* can be accomplished by heating at 95°-100° C for 15 min or 80° C for one hour. Samples must be in screw-cap tubes.

- Bring a heating block or metal pot filled with water into the BSC and bring to appropriate temperature
- Place *M. tuberculosis* sample to be killed into the block or boiling water
- After the appropriate time, turn off heat source and remove sample
- Wipe tube with Vesphene prior to removal from BSC
6. **Killing with chemical reagents**

Killing *M. tuberculosis* cells with chemical reagent occurs inside the BSC in a sealed tube. At least an equal volume of reagent is used to sterilize liquid cultures. All of the following reagents may be used to kill *M. tuberculosis* and will sterilize a culture by one minute after addition:

- 2.5% Vesphene
- Chloroform:methanol (1:1)
- 100% Formalin
- 4% Formaldehyde or paraformaldehyde

After addition of reagent, samples in sealed tubes are inverted to mix contents. Cover slips must be entirely submerged. After sample is killed, tube is wiped with Vesphene prior to removal from BSC and again prior to removal from BSL3.

7. **Transferring live *M. tuberculosis* between NWAF and BSL3**

Transfer live cultures in screw-capped shipping containers with O-rings containing absorbent material.

- Disinfect outside of culture tube
- Place in shipping container
- Disinfect outside of shipping container with Vesphene
- Remove from BSC
- Disinfect again with Vesphene before removing container from BSL3 facility
- Label with Biohazard sticker and contents
- Transfer immediately to BSL3 (or NWAF)
- Only open secondary container within BSC in BSL3

8. **Tissue culture**

Infected cell lines must be handled as if they were regular liquid TB cultures. Therefore, minimizing aerosols and avoiding spills is of utmost importance.

- Use flasks equipped with 0.2 μm filters when possible.
- Multiwell plates may be used if necessary. Always use breathable film sealed to the top of the plate to avoid spills.
- All plates and flasks must be clearly labeled with user's name and infectious agent.
• To image tissue culture dishes, a porous film is sealed to the top of the plate. Be sure to fully seal the film to the plate to prevent spilling. The sealed dish is then wiped with Vesphene and placed in a clean Tupperware container. The container is carefully wiped out of the BSC and taken to the microscope. The dish can be taken out of the container and imaged. Only one dish should be out of the container at any time.

• Aspirate liquid from tissue culture dishes using the aspirating pipet attached to a sidearm flask containing Vesphene. Be sure the filter is attached between the flask and the vacuum port. After aspirating, suck Vesphene into the aspirator and disassemble the aspirator and wipe out each piece.

9. Waste

• No waste can leave the BSL3 facility without being autoclaved

• All waste must be double bagged, marked with autoclave tape, and placed in covered plastic bins for autoclaving.

• Each worker is responsible for correctly bagging their own waste

• All personnel are responsible for autoclaving waste when the bin gets full. The Facility Director will ensure proper and safe disposal of waste

• Autoclaved waste must be removed from the clean side of the autoclave and disposed of in the red bins when the cycle is complete

• All users must be familiar with the proper use of the autoclave. Failure to use the autoclave correctly can result in malfunction. The Facility Director is to be notified immediately in the case of autoclave malfunction.

• Autoclaved waste is taken to room 230 Hildebrand Hall for disposal (entry code 889889).

Using equipment in the BSL3

The most important aspects of working with equipment in the BSL3 are to maintain secondary containment of pathogens and avoid generating aerosols. Specific concerns and protocols for common BSL3 laboratory equipment are listed below. Do not bring in new equipment unless authorized by the Facility Director. Failure to follow these guidelines for safe equipment usage will result in additional training or banning of the user from the BSL3.
1. Vortexer

**Vortexing generates aerosols.** All vortexing must be done inside the BSC and should be avoided if possible. The Vortexer is kept in a sealable container. Do not open this container outside the BSC.

- Turn on the BSC electrical outlets (on the control panel on the front of the BSC)
- Bring Vortexer container into the BSC
- Take Vortexer out of container and plug into BSC outlets
- Check Vortexer settings – vortex at the lowest setting possible
- **Only use Screw-cap tubes** for vortexing
- Wipe surface of sealed tube with Vesphene
- Vortex for the minimal amount of time possible
- When complete, wipe Vortexer with a damp Vesphene rag
- Place Vortexer back into the container
- Seal container and wipe out of the BSC

2. Table top centrifuge

**Centrifugation can generate aerosols.** Only screw-cap tubes can be centrifuged.

- Bring aerosol-tight centrifugation buckets into the BSC
- Wipe sealed tubes with Vesphene
- Place tubes in centrifugation buckets and seal bucket – check for O-ring
- **Be sure centrifuge is balanced**
- Wipe out buckets from BSC
- Centrifuge – **do not exceed 3500 rpm**
- Place buckets in BSC and open carefully
- Disassemble buckets and wipe out of BSC
- Rinse and dry buckets

3. Microcentrifuge

**Centrifugation can generate aerosols.** Only screw-cap tubes can be centrifuged.

- Bring aerosol-tight microcentrifuge rotor into the BSC
- Wipe sealed tubes with Vesphene
- Place tubes in microcentrifuge rotor and seal rotor
- **Be sure centrifuge is balanced**
• Wipe out rotor from BSC
• Attach rotor to microcentrifuge using the tool next to the microcentrifuge
• Centrifuge – do not exceed 14000 rpm
• Disassemble microcentrifuge and place rotor in BSC – open carefully
• Thoroughly clean the rotor with Vesphene and wipe out of BSC
• Rinse and dry rotor

4. Bath sonicator

Sonication generates aerosols. Only screw-cap tubes can be sonicated.

• Fill the bath with Vesphene
• Wipe out sealed tubes
• Place sealed tubes into the bath
• Turn on bath and sonicate
• When complete, immediately return tubes to BSC
• Turn off bath and empty sonicator

5. Electroporator

• Set Electroporator to desired settings
• Bring only the small portable electroporation unit into the BSC
• Place electroporation cuvette into electroporator (check for bubbles)
• Place moist Vesphene rag over electroporator
• Wipe out hands and begin electroporation
• If electroporator arcs – treat as a spill inside the hood

After electroporation, thoroughly clean electroporation unit and remove from BSC.

6. FastPrep bead beater

Bead beating generates aerosols. Only use O-ring, Bio101 screw-cap tubes. Only use the FastPrep bead beater in the BSC.

• Turn on BSC outlets (front of BSC)
• Move FastPrep into the BSC
• Plug into BSC outlet
• Insert tubes (O-ring, Bio101 screw-cap tube) into FastPrep
• Bead beat
• After bead beating, remove tubes and wipe entire FastPrep with Vesphene
• Remove from BSC
7. OD reader

All samples should be killed with Formalin prior to removal from BSC for OD reading. Formalin quickly kills *M. tb* without lysing the cells.

- Add 0.75 mL formalin to each cuvette
- Add 0.75 mL culture to each cuvette
- Carefully seal tubes with parafilm or cuvette lids
- Mix and wipe tubes out of the BSC
- Wipe each cuvette with 70% ethanol and dry
- After reading the OD, place samples back in the waste bin in the BSC
Emergency response

For ALL emergencies – call the Emergency Response Hotline (642-9090). They will contact the necessary personnel.

1. Biohazard spills inside the BSC

   • Immediately disinfect the area: Place dry towels on spill to absorb liquid. Soak these towels with Vesphene, working from the outside in towards the center of spill area.
   • Clean all affected areas within the BSC with disinfectant.
   • Leave UV light on in BSC after disinfecting, as usual. The BSC blower is never turned off during a spill.

A spill inside the BSC must be cleaned up immediately, but does not require any further notification.

2. Biohazard spill outside the BSC

DO NOT ATTEMPT TO CLEAN THE SPILL!

   • Hold breath, back away from spill
   • Tell everyone in the BSL3 to evacuate the facility immediately
   • If gown or shoe covers are contaminated, douse with Vesphene and remove them at the exit and leave on floor inside the BSL3
   • If clothing or gloves are contaminated, douse with Vesphene but keep them on
   • Once inside ante-room, remove protective equipment and any potentially contaminated clothing and place in a bag for autoclaving
   • Wash hands, face

All spills outside the BSC, regardless of size, must be reported immediately. Call the Emergency Response Hotline (642-9090), the Facility Director and the Biosafety Officer. If it is after normal working hours or the weekend, call the Emergency Response Hotline (642-9090).

Post signs so that others will not enter the contaminated area.

3. Decontamination of spill outside the BSC
Decontamination plan is established by the **Biosafety Officer**. No one can re-enter the facility without approval from the Biosafety Officer or other EH&S official. EH&S will make the determination for re-entry and has an emergency responder who will coordinate entry.

- After one hour, dress in protective clothing—rubber gloves, boots, disposable jumpsuit, head covering, and PAPR
- Pour disinfectant solution around spill and cover the area with paper towels soaked in disinfectant solution
- Let stand 30 minutes.
- Use paper towels to wipe up spill, working towards the center of the spill
- Swab area around spill, including floor, walls, counters, using paper towels soaked in disinfectant
- Dispose of all contaminated material used in cleanup in autoclave bags, and autoclave immediately

The Biosafety Officer will determine if gaseous decontamination is necessary.

After a spill, medical surveillance may be required for potentially affected workers.

4. **Equipment failure**

- Centrifuge rotor malfunction – ruptured centrifuge bowl
  - Treat as a spill outside the BSC and follow procedures outlined above
- BSC malfunction – red warning light and/or alarm signifies insufficient air flow
  - Cover and contain all infectious material, turn off vacuum, and close sash
  - Terminate work, notify all other workers to leave the room
  - Leave room, notify others not to enter by posting a sign
  - Notify the **Facility Director** and **Biosafety Officer** so that decontamination procedures can begin

5. **Fire alarm**

If there is not an immediate threat to your safety:

- Cover and contain all infectious material
- Close BSC sash
• Leave the BSL3 following normal procedures being careful to check for fire or smoke at doors
• Evacuate building using stairs
• Contact the Emergency Response Hotline (642-9090) or 911 to report a fire in the BSL3

If the main exit is blocked by fire:

• Go to emergency exit
• Remove PPE at the door
• Step onto the roof wait for fire department

If sprinklers are tripped:

• Cover and contain all infectious material
• Close BSC sash
• Leave the BSL3 following normal procedures being careful to check for fire or smoke at doors

6. Personal injury

• Needle sticks or cuts involving potential M. tuberculosis exposure
  o Allow wound to bleed – express the wound
  o Wash area with Vespheine
  o Wash affected area with soap and water
  o Call the Emergency Response Hotline (642-9090)
• Occupational injury such as exposure to hazardous chemicals
  o Report immediately to the Facility Director and Biosafety Officer
  o Seek medical attention

7. Room Pressure Failure

• If pressure sensors at the doors begin to alarm
  o Cover and contain all infectious material
  o Close BSC sash
  o Leave the BSL3 following normal procedures
  o Call the Emergency Response Hotline (642-9090)