GUIDELINES FOR USE OF THE ELIZABETH A. RICH BIOSAFETY LEVEL 3 FACILITY

CWRU SCHOOL OF MEDICINE
10TH FLOOR, BIOMEDICAL RESEARCH BUILDING

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DR. HENRY BOOM, DIRECTOR
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I. CWRU Biosafety Level 3 Organization, Equipment and Layout

A. Organization:

The Elizabeth A. Rich Biosafety Level 3 (BL-3) Facility of the Center for AIDS Research (CFAR) and its services are available to serve the needs of the Case Western Reserve University (CWRU) research community, and outside investigators who have established a collaborative working relationship with CWRU investigators.

BL-3 has a Director and a Manager/Research Assistant (RA). The BL-3 Advisory Group is composed of at least four members: the Director of the BL-3, the Director of the Department of Environmental Health and Safety, and at least two current BL-3 investigators. Meetings are organized by the BL-3 RA.

All activities in the BL-3 facility are administered according to the rules and regulations described in sections I and II of these guidelines. Section III is a copy of the CDC/NIH guidelines for BL-3 laboratories and is provided for your information.

The BL-3 Advisory Group meets as needed to discuss the operation of the facility, any new projects, and any safety issues that may have arisen. Project approval is obtained by submitting a protocol proposal to the BL-3 Director who will take the request to the BL-3 Advisory Group. Additional meetings are set up to decide on any necessary disciplinary actions. The BL-3 Advisory Group also discusses operation of the BL-3 facility and the scientific progress that has been made by the Principal Investigators (P.I.s) who use the facility. The P.I.s using the BL-3 facility are required to submit a summary of their progress upon request, and/or annually to the BL-3 Advisory Group for the purpose of the BL3 progress report.

The responsibilities of the RA include:
- Teaching new investigators and technicians methodologies in performing their research in BL-3 facility
- Maintaining records of BL-3 usage to process charge-back fees.
- Enforcing biosafety rules and regulations.
- Arranging for annual requirements such as respirator trainings, health screening (i.e. PPD or quantiferon test or health screening questionnaire submission), and exams
- Attending to all aspects of safety issues/emergencies that have arisen from work in BL-3.
- General maintenance of the BL-3 facility and its equipment.

The BL-3 RA is the user's point of contact for concerns regarding the BL-3 facility. The RA meets with the Core Director on a weekly basis (or as needed) to discuss issues that have been brought to their attention. The Core Director may be contacted directly by the user, if the RA cannot address the concerns.

B. Equipment and Layout:

The BL-3 facility is located on the 10th floor of the Biomedical Research Building, room 1007 (see map). The facility occupies 751 square feet and has three rooms used for biohazard...
work. Because the facility is for multiple users and its equipment is shared, cooperation and respect between users are imperatives. Space is limited and the facility should not be used as storage space. The -80° C freezer has space allotted for each lab and everyone must take care not to overflow into another lab’s space. All stored samples should be reviewed for disposal at least once every year and all outdated samples should be discarded.

The following is a list of equipment maintained in the facility:

<table>
<thead>
<tr>
<th>BSCs</th>
<th>Centrifuges</th>
<th>Incubators</th>
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</thead>
<tbody>
<tr>
<td>Four, 6-ft</td>
<td>Two table top</td>
<td>Two CO2</td>
</tr>
<tr>
<td>One, S3 Biosafety System</td>
<td>Two microcentrifuges</td>
<td>One roller incubator</td>
</tr>
<tr>
<td></td>
<td>One superspeed</td>
<td>Three shakers</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microscopes</th>
<th>Refrigerators / freezers</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>One inverted</td>
<td>Two refrigerators</td>
<td>S3 Cell Sorter</td>
</tr>
<tr>
<td>One light</td>
<td>One -80 freezer</td>
<td>Large &amp; small plate washers</td>
</tr>
<tr>
<td>One light, with computer</td>
<td></td>
<td>ELISA plate reader</td>
</tr>
<tr>
<td>One StereoScope</td>
<td></td>
<td>Sonicator</td>
</tr>
<tr>
<td>Colony counter system</td>
<td></td>
<td>Luminometer</td>
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<td></td>
<td></td>
<td>Thermocycler</td>
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Equipment not shown (in cabinets): two micro-centrifuges, tissue plate reader, thermocycler, heat blocks (2), large and small plate washers, microscopes: inverted (1), light (2), ultraviolet (1), stereo (2), column counter (1).
II. Procedures

A. Requirements of Users:

1. Users initially must take and pass a written examination based upon the BL-3 guidelines (including Parts I & II herein) before using the BL-3 facility, and subsequently pass a yearly refresher exam.

2. Users must have a series of two baseline PPD’s, or one quantiferon test before using the facility. Users must have a PPD skin test or quantiferon test yearly unless previously tested positive. If there is a history of positive PPD, a symptom questionnaire must be completed.

3. Users will complete a COVID-19 symptoms prescreen at the time of their health screen.

4. Users must receive a tour of the facility from the BL-3 RA.

5. Users must be signed off for on-the-job specific training at the bench by either the P.I. or a senior lab member designated by the P.I. before the user may work alone in the facility.

6. Users must be annually trained and successfully fitted, by their employer (CWRU Environmental Health and Safety, VA, UH, etc.), with a NIOSH-approved N95 half-face respirator.

7. Users and P.I.s must attend special BL-3 laboratory meetings when called. These will be impromptu meetings held on an "as needed" basis.

8. P.I.s must have projects approved by the Advisory Group before work by their laboratory staff begins in the BL-3 facility, and must submit progress reports yearly or upon request.

9. Each P.I. who wishes his/her lab group to use the BL-3 facility may elect either to be "BL-3 qualified" or to not be "BL-3 qualified". **Only** "BL-3 qualified" P.I.s may enter and use the BL-3 facility;
   a. Elect to be "BL-3 qualified"
      i. the P.I. meets all of the "requirements of users" listed in this section.
      ii. the P.I. **may** enter and use the BL-3 facility.
   b. Elect **not** to be "BL-3 qualified"
      i. the P.I. passes an exam, but does not take a yearly refresher exam, unless there are significant changes in the lab.
      ii. the P.I. signs a statement sheet indicating that he/she does not wish to become "BL-3 qualified".
      iii. the P.I. **may not** enter and use the BL-3 facility.

10. P.I.s must sign an agreement to abide by these guidelines for BL-3 facility use before using the facility.
B. BL-3 General Laboratory Practices:

1. All users are required to be familiar with the contents of the BL-3 Guidelines.
2. It is assumed that all users have prior experience in handling tissues, cell cultures, and pathogenic organisms. The BL-3 laboratory is not a training facility for these skills. No rotation students or summer students will be allowed to use the BL-3.
3. Users must be aware that safety is their primary responsibility and that users who violate safety guidelines potentially endanger not only themselves, but also their colleagues. Failure to follow the BL-3 Guidelines is grounds for loss of BL-3 laboratory use privileges. Offenders will be given a written warning after review by the Director and the relevant P.I. If the same violation recurs the user will be permanently barred from use of the facility.
4. Users should report any conditions perceived to be unsafe to the BL-3 RA. Any problem that cannot be resolved by the BL-3 RA, will be addressed by the Core Director and the BL-3 Advisory Group.
5. When entering the BL-3 facility, the user must complete the Sign In/Out sheet in the main anteroom. This is especially important in the event that an evacuation of the facility becomes necessary (i.e. fire alarm, pressure alarm). The Sign In/Out sheet indicates who is inside the BL-3.
6. Prepare carefully before starting an experiment in the facility. Think through your procedures before entering the BL-3 facility.
7. Open shoes or sandals are not permitted in the BL-3 laboratory. Covered shoes and socks or stockings must be worn.
8. The following items are prohibited in the BL-3 laboratory: food, beverages, chewing gum, makeup, lab-coat, and purses, as well as the use of any personal electronic and auxiliary devices such as cell phones, MP3 players and headphones/earbuds.
9. Paper and pens may be brought into the BL-3 laboratory, but cannot leave.
10. BL-3 facility users supply their own tissue culture materials and plastic-ware. Each lab is assigned an identification color and must label their supplies and samples with tape in the assigned color. This includes items in the refrigerator, freezers, drawers and shelves.
11. Items in the incubators and rotators must be labeled with the name of the lab, user and the date. All labs that keep cultures in the incubators/rotators are required to indicate that they have inspected their cultures by initialing and dating the log sheets on the respective incubators. Because fungus problems tend to recur in a shared facility, cultures should be visually checked twice a week. The BL-3 RA conducts spot checks weekly. If a culture goes more than a week without being checked by the user, the user will be notified. If the user does nothing within the next 24 hours, the RA will discard the culture. If a person has cultures discarded twice by the BL-3 RA, the person will have his/her privileges of BL-3 facility use suspended. Users who cannot fulfill this requirement must ask someone else to do it for them.
12. To avoid the possibility of spreading a fungal contamination follow this procedure: Visually inspect all cultures thoroughly before any manipulation. If fungus is found in a culture, do not open the plate or flask. Put the plate or flask into a ziplock bag and place that bag into doubled biohazard bags. Secure the
biohazard bags with autoclave tape and autoclave the contaminated material immediately. The RA will inspect incubators weekly. If a contaminated culture is found, it will be discarded and the culture's owner notified. If you see a contaminated culture that belongs to another lab, notify the RA immediately.

15. It is recommended that only filtered pipette tips be used in the facility.

16. It is recommended that fungizone be used in culture media whenever possible.

17. Only filter-top flasks may be used in the BL-3 laboratory. Loose-top flasks or tubes are not permitted. Vented filter-top flasks will fail only when the culture is tipped and the filter gets wet.

18. The waste container of the ELISA platewasher should contain 10% bleach solution and its fluids must be treated as biohazardous waste. Allow the fluid waste to remain in the bleach solution for at least five minutes before washing the mixture down the sink with a copious amount of water. The waste container should be emptied after each use.

19. Safety cups must be used on centrifuge rotors at all times. 50mL and 15mL conical tubes that are used for balancing the centrifuge must be discarded after each use.

20. All manipulations of cultures must be done in a biosafety cabinet (hood).

21. All trash and biohazardous waste leaving the facility must be autoclaved for one hour using the LAB WASTE cycle. Users must be trained for the autoclave before using. Only one door of the dual-port autoclave may be open at any one time. After a sterilization cycle, items to be removed from the facility must be removed from the outside door (NOE, Non Operating End). Once removed the outer door should be closed and sealed to allow the inner door (OE, Operating End) to be opened. Under no circumstances should the outer autoclave door be opened after the inner autoclave door has been opened, unless the autoclave has run a full (1 hr) cycle. Therefore, the trash must be removed from the outside first. Do not seal the inner door of the autoclave until the autoclave is ready to be run again. This prevents the outer autoclave door from being opened until it has been sterilized again.

22. Troughs with contents may be decontaminated alone by the following process: Allow trough contents to soak in 10 - 20% bleach solution for 20 minutes. Drain the bleach solution while flushing with running water then autoclave using the “TROUGHS” cycle. Pipette troughs must be loaded and removed through the inner door and then emptied immediately into a sharps bin to prevent a sharps hazard when they have cooled.

23. If the autoclave is ever broken, the following procedures will be followed. If the autoclave can be fixed within a few weeks, red bags will be stored in one of the hoods (depending on the volume) until the autoclave is fixed. The contents of the troughs will be emptied into sharps boxes and the troughs will be rinsed and sprayed with 10% bleach before being reused. In this case, all items must be decontaminated with 10% bleach before being placed into red biohazard bags. The bags will be sprayed down and brought out to be autoclaved. The sharps boxes containing the contents of the troughs will also be sprayed down and brought out to be autoclaved.
C. Entry/Exit Procedures:

Personnel:

1. The hallway door of the BL-3 facility is always locked. Each user of the BL-3 must enter the facility by scanning his/her CWRU identification card. Entry into each section of the facility before the BL-3 lab must be staggered to be in compliance with the University’s COVID precautions.

2. Before every entry into the BL-3 lab, the user should sign into the logbook in the main anteroom.

3. Walk over the "Tacky Mat" so that particulate matter is removed from the bottoms of shoes.

4. In the locker room change from your street clothes into scrubs, and hang your clothes in your assigned locker. Then put on a complete set of personal protective equipment (PPE) over the scrubs. There are signs posted in the locker room with specific instructions for donning. It is important that the correct procedure is maintained in order to minimize the risk of exposure.

A complete set of PPE consists of the following:

a. Respiratory protection:
   - N95: The user must have been previously fitted and trained in the use of a NIOSH-approved N95 respirator, and must don the respirator which he/she was fitted and trained to use.
   - PAPR: These are located in the BL-3 ante-room and should be donned last. These will remain in the anteroom as they are reusable and must be sprayed with disinfectant upon exiting the lab.

b. Tyvek coveralls. These must be discarded after each use.

c. Two pairs of gloves. The first pair with extended cuffs, taped to your overalls for complete coverage; and the second, a pair of regular exam gloves that can be quickly changed if necessary.

d. Eye protection – disposable face shields or plastic goggles, to be discarded or decontaminated after each use, must be worn upon entry into the lab. These are available in the BL-3 ante-room and are only used with an N95 (not needed with a PAPR)

5. When exiting, remove all PPE in the BL-3 ante-room before proceeding to the locker room. Signs with specific instructions for doffing are posted in the ante-room and must be followed closely to minimize any risk of exposure. PAPR’s must be sprayed and wiped with LpH upon removal. PPE must never be worn outside of the BL3 ante-room.

6. Under normal circumstances, your scrubs should not be contaminated, and you will change from your scrubs back into street clothes in the locker room. Leave your scrubs in your labeled locker to be reused. Once these scrubs are dirty, put them in the laundry bag in the locker marked “scrub laundry”. The Laundry bag will be emptied weekly or whenever the bag reaches capacity, whichever comes
first. Only scrubs placed in the laundry bag will be taken. The RA will not “sweep” your lockers for dirty laundry.

7. **All personnel must wash their hands in the locker room bathroom before leaving the facility.**

8. Walk over the tacky mat to enter the main anteroom.

9. Complete the Sign In/Out log to indicate the time leaving the facility.

10. In order to comply with current safety guidelines and for the safety and privacy of all users, we ask that users adopt a staggered entry into the rooms for donning and doffing. We propose that each user wait 10 minutes after another user before entering the facility, and the same before exiting the BL-3.

### D. Materials Handling:

1. Corrugated cardboard is not permitted inside of the BL-3 laboratory.

2. Paper must **never** be removed from the BL-3 lab without sterilization.

3. Equipment that must leave the BL-3 facility must be decontaminated with an appropriate disinfectant. Check with the BL-3 RA before carrying out this procedure. A *fresh 10% bleach solution* must be used to wipe containers that are leaving BL-3.

4. Samples may be taken from the BL-3 lab for further analysis with the permission of the BL-3 RA or the BL-3 Lab Director. Taking samples from the BL-3 lab without permission is grounds for loss of BL-3 lab use privileges. The following describes the procedure that must be utilized for the safe transfer of culture-derived material from the BL-3 lab to the outside environment:

   - **a.** The PI or user will consult with the RA or the BL-3 Lab Director about the transfer and obtain his/her approval of the transfer procedure **before** it is conducted.

   - **b.** The primary container (i.e., vial, conical tube, bottle) holding the material will be secured (i.e., the cap (s) or top(s) will be tightened) to prevent leakage.

   - **c.** The primary container will be placed inside a leak-proof secondary container that is also resistant to punctures (i.e., commercially available biohazard mailers and shippers are suitable for use as secondary containers). The secondary container will be secured (i.e., the cap(s) or top(s) will be tightened) for the containment of any leakage that might occur from the primary container during the transfer.

   - **d.** The outside of the secondary container will be completely wiped down with a fresh solution of *10% bleach immediately prior to the container's removal from the BL-3 lab.

   - **e.** The secondary container will not be opened outside of the BL-3 facility prior to the container's arrival at a suitable storage facility (i.e., freezer) or biosafety cabinet, if the sample has been thoroughly decontaminated, or to another BL3 - lab, if not.

Because leakage or breakage of the primary container might have occurred during the transfer, the secondary container should be opened only within a biosafety
cabinet. The owner of the culture-derived material assumes all responsibility for the safe handling of the material once the containers leave the BL-3 facility.

5. Reusable items, such as pipette tip boxes, must be autoclaved before leaving the facility. These items must be retrieved through the autoclave's door outside of the BL-3 facility.

E. Working in a Tissue Culture Hood (Biosafety Cabinet):

1. Concentrate on your work and use common sense to be safe.

2. Change outer exam gloves frequently, discarding them inside the BSC, when working with infectious materials and when the gloves become contaminated, torn or punctured.

3. Do not clutter the hood because this interferes with proper airflow.

4. Before starting your work, the following items should be set-up in the hood:
   a. Line the small bucket in the hood with a red biohazard bag. This bucket is for garbage generated in the hood such as test tubes, flasks, gloves, and materials used to wipe up small amounts of fluid from the hood's work surface.
   c. Fill a pipette trough approximately 1/3 full with between 15 - 20% bleach solution. All pipette tips, serological pipettes, cell scrapers, syringe-filter combinations, and transfer pipettes must be discarded into this container. Serological pipettes and transfer pipettes should be filled with bleach before being discarded into the container. **Do not fill the pipette container more than 1/2 full with pipettes because the pipettes must be fully immersed in the bleach solution. If the user has large amounts of waste, multiple troughs should be used as overfilling could create a hazardous situation.**

   The trough can be used to dispose of small amounts of culture medium, however if you need to dispose of larger volumes, please prepare a separate container so that your final volume to be disposed contains 10 - 15% bleach.

   d. If needed, use a two flask system to aspirate liquid. Fill both the collection and overflow flasks to the lower mark with bleach. Do not fill the flask past the upper fill line and take care not to aspirate the liquid into the vacuum line. A hydrophobic or HEPA filter **must** be used between the overflow flask and the vacuum line.

5. All SHARPS (i.e., glass slides, coverslips, needles) **must** be disposed of in an approved red SHARPS container that the user must place inside of the hood. When this container becomes filled or at the end of use, it must be securely closed and a piece of autoclave tape must be applied over the lid. The container must remain in the hood until it is removed to be autoclaved.

6. In case a glass is broken, use the forceps that are kept on the shelf above the sink to pick up and dispose of it. **DO NOT pick up broken glass with your hands!**

7. Glass beads that are used in MTB experiments **must** remain inside of the container in which they are used to break up clumps of the bacteria. After the bacteria have been removed from the container, the container must be filled with
bleach solution (i.e., use a serological pipette or a pipette tip to take up bleach from the pipette trough and dispense the bleach into the container), tightly recapped, and disposed into the pipette trough.

8. Spray all materials coming out of the hood with either the currently approved disinfecting solution or a 10% bleach solution. If any bleach is spilled in the hood, it should be cleaned up so it won’t corrode the hood.

9. Any spill inside the hood must be immediately treated with an approved disinfectant and wiped up with absorbent tissues.

10. Clean up when you're finished working in the hood:
   a. No known hazardous materials should be placed in the garbage can outside of the hood. This can is for solid waste that has not been inside the hood
   b. The beaker and flask containing bleach solution with waste must sit for 20 minutes before discarding the liquid waste down the sink. All liquid waste discarded in this manner must be flushed by a copious amount of water (at least double the volume).
   c. After pouring the liquid waste into the sink, rinse the containers out with water, and decontaminate using the procedures for reusable glass and plastic ware described below.
   d. All reusable glass and plasticware used in the hood should be immersed completely in the carboy containing a freshly-made approved disinfectant or *10% bleach solution. The items must soak for at least 20 minutes. After soaking the reusable items the carboy must be emptied and rinsed. The reusable items must also be thoroughly rinsed and then hand dried with paper towels before replacing them on the shelves.
   e. For pipette troughs: Make sure all items are completely submerged in 10 - 20% bleach. Filled troughs must soak for 20 minutes. After 20 minutes, pour the disinfectant in the sink, flushing continuously with running water. Autoclave the trough and contents using the QUICK cycle (10 minutes). Once the cycle is complete, remove the trough and dump the contents in the red sharps bin. Rinse and reshelve the trough for future use. The RA will dispose of the SHARPS bin once it is full.
   f. Wipe down the interior work surfaces of the hood with an approved disinfectant or *10% bleach solution and place all wipe-up materials in the red biohazard bag inside the hood. To prevent corrosion, please do a final wipe down with 70% ethanol provided in spray containers. PLEASE WAIT AT LEAST 5 MINUTES before wiping with ethanol. Remove and dispose of the outer pair of gloves in the biohazard bag inside the hood. Replace the outer pair of gloves. While keeping the red biohazard bag inside of the hood, loosely tape the bag closed with autoclave tape and spray the outside of the bag with *10% bleach before carefully removing and then placing it in a second biohazard bag. Autoclave on DRY cycle.
   h. The hood blower must be left "on" at all times.

11. If the air balance has been disturbed, the alarm will activate on the biohoods as well as the wall panels. Should this occur while working, users should take care to contain all work in the biosafety hood. Any particles that escape from the
hood, could also escape from the BL-3 when the negative airflow is disrupted. All work should be stopped, the outer pair of gloves should be slowly removed, and the user should walk away from the hood taking care not to disrupt the airflow barrier at the interface of the biohood. The user should check the panel to see if negative pressure has been restored. This generally takes less than a minute unless there is a more serious problem. If the pressure has been restored the user may return to his/her work once the alarm has stopped. If the pressure remains positive after one minute, the user must spray their coveralls with 10% bleach solution and exit the lab immediately.

10. Should these emergencies in the BL-3 take place, a sign must be posted to prohibit other users from entering the facility. Contact the BL-3 RA or Director to notify them of the situation. Wait for instructions before re-entering the lab.

12. All users are responsible for the general orderliness of the BL-3 facility. The RA will check the lab for compliance, change the tacky mat, and order supplies, but you are responsible for daily tasks and alerting the RA if certain supplies need to be ordered.

**BioRad S3 Cell Sorter**

1. All users must be trained BEFORE using the sorter. Please contact the BL3 RA to arrange training.

2. Users must log into the iLabs system to reserve the sorter. Actual usage must be logged into the user log provided in the lab.

3. Users must use face shields, not safety goggles, while using the sorter.

4. The S3 Containment system is a Biosafety container and must be treated as such. No **OPEN** samples should be removed from this bio-hood.

**F. Spills:**

1. In the event of an MTB spill outside of a hood, notify the other workers inside the BL-3 lab. **Everybody must exit the facility.**

2. Dam off the spill with paper towels and post a warning sign at the entryway to the spill area. NO ONE should enter the lab during this time.

3. Pouring carefully, saturate the spill with bleach, leave the lab, and let the bleach sit for **at least 2 hours.**

4. Notify the BL-3 Research Assistant or the Director of the BL-3 facility.

5. After 2 hours, put on clean Personal Protection Equipment (PPE) to return to the spill.

6. Obtain the "Red Z" Fluid Control Solidifier container from the spill kit stored inside the cabinet located next to the window. Sprinkle the Red Z powder evenly over the spill. Allow sufficient time for solidification of the spill to occur (usually 5-10 minutes; sprinkle more Red Z powder over the spill if fluid remains after this time).

7. Remove the solidified spill with a scoop (found in the spill kit), or with paper towels and dispose of all of the waste inside of doubled biohazard bags.
8. Clean the area of the spill with the SaniZide Plus Germicidal Solution that is stored in the spill kit. **Allow the disinfectant to remain on the spill area for at least 10 minutes.** Wipe the area dry with paper towels, discarding them into the doubled biohazard bags.

9. Tie the biohazard bags loosely with autoclave tape, place the bags inside the autoclave and immediately sterilize them for one hour (DRY cycle on the autoclave touchpad).

10. An incident report **must** be written for any spill that occurs outside of a BL-3 lab hood.

G. **Exposures:**

Please note any exposure must also be handled as a spill outside of the BSC. All other users must be asked to leave the lab immediately and a sign must be posted to prohibit entry until after the spill has been cleaned or the exposure is completely dealt with. Exposures must be handled in the following order:

1. Exposure to eyes, skin or any mucous membranes: Immediately remove goggles or clothing and wash the involved area with copious amounts of water. There is an eyewash station located by the sink in the BL-3. Proceed to the emergency station in the **ante-room** and follow the instructions for removal of contaminated PPE/clothing. Goggles and respirator must be replaced immediately before returning to clean the spill.

2. Spills - this should be handled next to avoid continued exposure. Details are described in the previous section.

3. Exposure to tyvek suit: Remove suit immediately. Discard and seal in any red biohazard bag to be autoclaved later. Proceed immediately to the ante-room. Leave scrubs in the autoclave bag in the ante room and proceed to the locker room in the clean scrubs provided in the ante-room emergency kit.

4. Exposure to any clothing other than tyvek suit, ie. scrubs: In the rare event that scrubs become contaminated, proceed to the emergency station in the **freezer room** immediately and follow the instructions for removal of contaminated PPE/clothing. Leaving the respirator and goggles/face-shield in place, remove contaminated scrubs as quickly as possible and place them in the designated bag. A pair of scissors will be available for cutting the top to avoid pulling it over the face during removal. A clean set of scrubs will be placed in a ziploc container and placed in this area for such emergencies. Wipe any exposed areas of skin gently with soap and water before proceeding to the anteroom to remove the respirator and goggles/face-shield. Proceed to the locker room and again, wipe any exposed areas with soap and water before putting on street clothing. If the user feels it is absolutely necessary, he/she may use the shower after wiping.

5. Upon exiting the lab, immediately report the incident to the BL-3 RA, to your supervisor and to the University Health Service. During regular working hours, go directly to the University Health Service, located at 2145 Adelbert Road or call the needle-stick hotline (368-6635). Identify yourself as a BL-3 facility user. You will be required to complete an incident report upon your visit to the University Health Service.
If this is not during regular working hours, report the incident to the BL3 RA and your supervisor and complete the incident report online at

H. Remarks:
The BL-3 laboratory is a core facility that is used by many people. In order for things to work smoothly, everyone must cooperate and respect other users. Using the BL-3 laboratory is a privilege that can be suspended. Safety is the primary concern for all users. All users rely on each other to ensure their own and other’s safety. Remember that only unity in practice assures safety in the BL-3 laboratory.

I. Safety Notes:

1. Permissible exposure times from UV light in a biosafety cabinet are between 28 seconds for hand level exposures at the cabinet face and 1.4 hours at general eye level in the center of the room (Burgener 2006). The cabinet sash, eyewear and coveralls lengthen the allowed exposure time, but do not block it completely.

2. Limitations of UV Light in Biological Safety Cabinets:
The primary means of sterilizing the air in the BL-3 is the use of the hoods and the negative pressure in the room. The hoods and negative pressure systems both utilize HEPA filters. The primary means of sterilizing surfaces is the use of 10% bleach or LpH. UV light inside the hoods is an extra measure that can kill TB (Meechan et al. 2006), but it should not be relied upon as the primary means of disinfection for many reasons (Burgener 2006). In a dynamic air stream (e.g., biological safety cabinet) particles do not come close enough to the UV source to be affected for a sufficient period of time. Microorganisms beneath dust particles or beneath the work surface are not affected by the UV irradiation. This includes dust that accumulates on the bulb itself; UV bulbs should be cleaned weekly with ethanol. Humidity, temperature and the age of the UV bulbs also affect output. Below 70% humidity, between 77-80°F, and bulbs less than one year old are optimal. The effectiveness is also reduced with distance from the bulb. At the minimum acceptable irradiance in a biosafety cabinet, it takes 12.5 minutes to be germicidal for spore forming organisms (Burgener 2006). TB may be two to three times less sensitive to UV irradiation than gram-negative bacteria (Collins 1971 and David 1973).

3. Disinfectants:
a. LpH. EPA approved. Effective within 10 minutes contact time. Diluted 1:256, one half ounce in one gallon. The diluted solution is good for 14 days. Effective for both MTB and SARS CoV2.
b. Bleach. EPA approved. Effective within 5 minutes contact time. Bleach is an approved disinfectant for both MTB and SARS CoV2. A 10% solution must be made fresh daily. **Bleach can corrode metals, such as the biohoods, so it is recommended that after disinfecting with bleach, these be wiped with 70% ethanol after 5 waiting minutes.**
III. CDC/ NIH Guidelines for BL-3 Laboratories:

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. Laboratory Biosafety Level Criteria: BSL-3 39

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:
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   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. (See Appendix G.)

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. Laboratory Biosafety Level Criteria: BSL-3 41
   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 wear 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. 42 Biosafety in Microbiological and Biomedical Laboratories
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. Laboratory Biosafety Level Criteria: BSL-3 43
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. 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. 44 Biosafety in Microbiological and Biomedical Laboratories
   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. 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.

10. 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.

11. 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).

12. 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.

13. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.

14. 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. Laboratory Biosafety Level Criteria: BSL-4 45

15. 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.


IV. Appendices

Appendix A: Current Directors and Members

The BL-3 laboratory is a core facility of the Center for AIDS Research (CFAR) with Dr. Jonathan Karn as Director. Dr. Henry Boom is Lab Director. The BL-3 Advisory Group includes Marc Rubin, Dr. Richard Silver, Dr. Supriya Shukla and Dr. David Canaday. The BL-3 Research Assistant is Sophia Masters Onwuzulike.

Appendix B: Current List of User Labs and their Projects

Boom Lab
- TBRU funded project, MTB TLR signaling.
Silver Lab  
- NIH funded RO1 on contact mediated host resistance to TB

Harding Lab  
- NIH funded RO1 on TLRs in pathogenesis of MTB

Sekaly Lab  
- NIH funded RO1 MTB virulence factors

Karn Lab  
- NIH funded RO1 MTB virulence factors

Appendix C: Current List of User Labs and Their Assigned Identification Colors

Core - white  
Nguyen - blue  
Boom - pink  
Silver - green  
Harding - orange  
Sekaly - purple  
Karn - yellow

Appendix D: General Guidelines for Maintenance/Repair Procedures Performed within the BL-3 Laboratory by Personnel Other than BL-3 Lab Users

1. All BL-3 lab users will be informed by the Research Assistant of the planned maintenance/repair procedure at least 24 hours prior to the procedure's scheduled appointment. If the procedure is to be done because of an emergency, the RA will inform all BL-3 lab users as soon as possible.
2. The RA will post a sign on the outside door of the facility informing of the shutdown.
3. The RA will prohibit all manipulations of pathogens inside of the BL-3 lab at least two hours prior to the scheduled time of the maintenance/repair procedure, and absolutely no manipulation of any pathogen will be allowed during the maintenance/repair procedure. The ideal prohibition includes the preceding night.
4. The RA must decontaminate the equipment to be serviced before the maintenance/repair work is allowed to proceed. The decontamination procedure must be made with an approved
disinfectant that is active against all of the pathogens being manipulated within the facility. The decontamination must be documented.

5. Maintenance/repair personnel entering the BL-3 lab must don personal protective equipment (PPE) that is at least as protective as that being used by the BL-3 lab users. Presently, this consists of Tyvek coveralls with attached hood and boots, two pairs of gloves (The first pair with extended cuffs taped to overalls for complete coverage; and the second pair that can be quickly be changed if necessary), fitted N95 NIOSH-approved respirator, and protective eyewear.

6. The RA must encourage the maintenance/repair personnel to take into the BL-3 lab the minimum amount of tools and instruments he/she will need to perform the required task. Before entering the BL-3 lab, the RA must inform the maintenance/repair personnel of the decontamination procedure that will be used upon those tools and instruments before they're allowed to exit the BL-3 lab.

7. The RA must accompany the maintenance/repair personnel into the BL-3 lab.

8. The RA is responsible for supervising the safe and proper egress from the BL-3 lab. This responsibility includes the proper decontamination of all tools and instruments, and the proper doffing and disposal of PPE before the items and personnel are allowed to exit the BL-3 facility. The maintenance/repair personnel must wash his/her/their hands with warm water and antimicrobial hand soap inside the locker room bathroom before they leave the core facility.

Appendix E: Procedure for Manipulating a New Pathogen within the Biosafety Level 3 Facility

The Biosafety Level 3 (BL-3) Facility may only be used for the manipulation of pathogens that have been assigned to or below Risk Group 3 by the National Institutes of Health, Office of Biotechnology Activities. Pathogens that have been assigned to Risk Group 4 are strictly prohibited from entering the BL-3 Facility.

An investigator wishing to manipulate a pathogen that has not been previously manipulated within the BL-3 Facility must:

1. Submit a written protocol proposal for the approval of the BL-3 Advisory Group; the protocol must include:
   a. the name and description of the pathogen
   b. a description of the organism's pathogenicity in humans
   c. a list of the available diagnostic tests and vaccine specific for the pathogen
   d. a description of the procedures that will be used in the manipulation of the pathogen
   e. A list of equipment needed
   f. a description of the procedure to be used for the disposal of all waste that is generated by the manipulation of the pathogen
g. a list of references relating to the laboratory manipulation of the pathogen

2. Agree to present an in-service to all of the users of the BL-3 Facility for the purposes of informing them of the new pathogen, the procedures that will be used within the Facility for its manipulation, and for addressing any questions and/or concerns the facility users may have.

The Facility Research Assistant (RA) will acquire supplementary information regarding the manipulation of the pathogen as needed, including relevant guidelines published by the CDC, the National Institutes of Health (NIH), and information from outside experts who are experienced with handling of the pathogen. The RA must also participate in the in-service that is presented to the users of the Facility.

When the protocol proposal has been approved by the BL-3 Advisory Group, the BL-3 Facility management will:
1. Formulate and institute a program for the surveillance of facility users for exposure to the pathogen.
2. Amend the current versions of the Guidelines for the Use of the BL-3 Core Facility, and the Blood Borne Pathogens Exposure Control Plan to include the surveillance program, and the approved guideline and protocol for the safe handling of the new pathogen. The amended documents will be distributed to all users of the Facility.

**Manipulation of the pathogen within the BL-3 Facility will not be allowed to proceed before all of the above actions have been successfully completed.**