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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I. CWRU Biosafety Level 3 Organization, Equipment and Layout ..............................................3
   A. Organization..................................................................................................................3
   B. Equipment and Layout...............................................................................................4

II. Procedures ..................................................................................................................................6
   A. Requirements of Users .................................................................................................6
   B. BL-3 General Laboratory Practices...............................................................................6
   C. Entry/Exit Procedures....................................................................................................8
   D. Working in a Tissue Culture Hood................................................................................10
   E. Spills ............................................................................................................................. 12
   F. Exposures....................................................................................................................... 14
   G. Remarks ...................................................................................................................... 14
   H. Safety Notes.................................................................................................................. 14

III. CDC\NIH Guidelines for BL-3 Laboratories ........................................................................ 16
   A. Standard Microbiological Practices ............................................................................. 16
   B. Special Practices........................................................................................................... 16
   C. Safety Equipment (Primary Barriers)........................................................................... 18
   D. Laboratory Facilities (Secondary Barriers).................................................................. 18

IV. Appendices..............................................................................................................................20
   A. Current Directors and Members....................................................................................20
   B. Current List of User Labs, Their Projects and Their Priority Scores............................20
   C. Current List of User Labs and Their Assigned Identification Colors.........................21
   D. General Guidelines for Maintenance/Repair Procedures Performed within the BL-3 Laboratory by Personnel Other than BL-3 Lab Users.............................................21
   E. Procedure for Manipulating a New Pathogen within the Biosafety Level 3 Facility…22

V. Exposure Control Plan for Bloodborne Pathogens ...............................................................23
I. CWRU Biosafety Level 3 Organization, Equipment and Layout

A. Organization:

The Elizabeth A. Rich Biosafety Level 3 (BL-3) Facility of Core E of 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.

Core E has a Director and a Manager/Research Assistant (RA). The BL-3 Advisory Group is composed of four members, the Director of Core E, and the Director of the Department of Occupational and Environmental Safety, and two current BL-3 investigators. Meetings are organized by the Core 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 bi-annually for regular discussion and as needed to discuss the operation of the facility, any new projects, and any safety issues that may have arisen. The bi-annual meetings will be held in May and November. All new projects will be reviewed and assigned a priority score. Project approval is obtained by submitting a summary of the project to the BL-3 Director who will take the request to the BL-3 Advisory Group. Additional meetings will be 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 annually to the BL-3 Advisory Group for the purpose of Core E 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 and maintaining records of HIV and PPD testing.
- 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.
- Autoclaving all trash, emptying the carboy and changing the tacky mat.

The Core 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 to discuss issues that have been brought to their attention.

A priority for usage has been established by the BL-3 Advisory Group, for use of the facility. The priority scores determine which types of projects have the highest priority (1) and which have the lowest priority (3). These scores are used in instances in which all of the hood spaces in the BL-3 facility are reserved. The person with the lowest priority score is asked to
reschedule usage time. Individuals with lower priority scores are given at least 24 hours notice when asking him/her to reschedule.

1A: AIDS-related, NIH-funded or AmFAR-funded projects. (includes: TBRU).

1B: Non-AIDS, NIH-funded work.

2: AIDS-related, non-funded pilot projects.

3: Non-AIDS-related, non-funded pilot projects.

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>Hoods</th>
<th>Centrifuges</th>
<th>Incubators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Five, 6-ft</td>
<td>Two table top</td>
<td>Three CO₂</td>
</tr>
<tr>
<td>One, 4-ft</td>
<td>Two microcentrifuges</td>
<td>One roller incubator</td>
</tr>
<tr>
<td></td>
<td>One superspeed</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microscopes</th>
<th>Refrigerators / freezers</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>One light</td>
<td>Two refrigerators</td>
<td>ELISAPlate reader&amp;washer</td>
</tr>
<tr>
<td>One inverted</td>
<td>One -80 freezers</td>
<td></td>
</tr>
<tr>
<td>One light, with computer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One StereoScope</td>
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</tbody>
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It is mandatory that potential users consult the RA before their initial use of any equipment.
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) and the Bloodborne Pathogens Exposure Control Plan before using the BL-3 facility, and subsequently pass a yearly refresher exam.
2. Users must have a baseline HIV and PPD test before using the facility.
3. 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.
4. Users must have a PPD skin test yearly unless previously tested positive.
5. Users must have an HIV test annually.
6. Users must be annually trained and successfully fitted, by the CWRU Department of Occupational and Environmental Safety, 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.
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" people working for the P.I. 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" the initial written exam given to all users of the facility, but does not take yearly refresher exam.
      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 signify their agreement to abide by these guidelines for BL-3 facility use by signing the attached signature sheet 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 that they perceive to be unsafe to the Core RA. Any problem that cannot be resolved, 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 antechamber. This is especially important in the event that an evacuation of the facility becomes necessary (i.e. fire alarm, pressure alarm) or if the UV lights are going to be turned "on". 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. Sandals are not permitted in the BL-3 laboratory. Covered shoes and socks or stockings must be worn.

8. Plastic-wear must be substituted for glassware whenever possible. All culture medium must be ordered in disposable plastic containers.

9. The following items are prohibited in the BL-3 laboratory: scissors, scalpels, food, beverages, chewing gum, makeup, lab-coat, purses and notebooks.

10. The use of special needles MTB experiments is possible only if absolutely required for a specific procedure. Such procedures must be discussed and approved by the Director and the BL-3 Advisory Group before they are initiated.

11. Paper and pens may be brought into the BL-3 laboratory, but cannot leave.

12. 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.

13. Items in the incubators must be labeled with the name of the user and the date. All labs that keep cultures in the incubators are required to indicate that they have inspected their cultures by initialing and dating the log sheets that are attached to the front of the incubators that contain the cultures. Because fungus problems tend to recur in a shared facility, cultures should be visually checked three times a week, including Monday and Friday. The BL-3 RA also will check all cultures on Friday and conduct spot checks. 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 Research Assistant, 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.

14. 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 if the autoclave is available. Otherwise, notify the BL-3 RA that the bag contains fungus and needs to be autoclaved as soon as possible. The BL-3 RA will inspect incubators twice a week. 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 and it will be discarded and the lab notified.

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 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 bleach and its fluids must be treated as biohazardous waste. Allow the fluid waste to remain in the bleach for at least two 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 (tissue culture hood).
21. All trash and biohazardous waste leaving the facility must be autoclaved for one hour (cycle 3 for solids or 4 for liquids on the autoclave touchpad). Only one door of the dual-port autoclave may be open at any one time. After a sterilization cycle, the autoclave door that is outside of the BL-3 facility must be opened and closed before the autoclave door that is inside of the BL-3 facility is opened. Under no circumstances should the outer autoclave door be opened after the inner autoclave door has been opened.

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 using his/her CWRU identification card in the key-card lock installed on that door.
2. Before every entry into the BL-3 lab, the user should sign into the logbook in the entryway. After signing in, the sign on the door should be changed to Red stating “BL-3 OCCUPIED – NO OVERHEAD UV’S.
3. Walk over the "Tacky Mat" so that particulate matter is removed from the bottoms of shoes.
4. Before every entry into the BL-3 lab, users must put on a complete set of personal protective equipment (PPE) in the locker room. A complete set of PPE consists of the following:
   a. Two pairs of gloves (One long pair under the coveralls and one pair of
regular exam gloves that can be quickly changed over the coverall cuffs)

b. Tyvek coveralls (coveralls may be worn 3 times unless contaminated.
Mark with your name and a slash mark each time worn and hang in the
BL-3 ante-room)

c. Shoe covers (over covered shoes and socks or stockings)

d. Eye protection – Plastic glasses are re-useable; these should be labeled and
left in the BL-3 ante-room

e. Respiratory protection: The user must have been previously fitted and
trained in the use of a NIOSH-approved N95 respirator, and must don the
respirator with which he/she was fitted and trained to use.

When exiting, remove all PPE in the BL-3 ante-room. PPE must never be worn
outside of the facility.

5. All personnel must always wash their hands in the locker room before leaving the
facility.

6. The Sign In/Out sheet must be completely filled out upon entering and exiting the
BL-3 facility. Before every exit from the BL-3 lab, the user should check the
facility for other users. If no one else is present in the facility (i.e. the user is the
last one to exit the facility), the user should check the logbook to be certain that
no one else is in the laboratory, flip the Green sign stating “BL-3 EMPTY-
OVERHEAD UV’S ON”, and flip the switch to turn on the overhead UV lights.
The switch is located immediately to the right of the door to the BL-2. The
overhead lights should remain on at least one hour after use of the BL-3, but may
remain on overnight.

Should the user ever be working in the facility and the overhead UV’s be
flipped on in error, the user should stop working immediately, go to the
locker room and remove PPE quickly, and then exit through the door to
the entryway (where the logbook is located) as the opening of this door is
the only way to turn off the overhead UV lights (they are not turned off by
the switch that turns them on). Alternatively, the user may call someone
who has access to the facility to come and open the entryway door to turn
off the lights, however, they must ensure that assistance will come
immediately to avoid exposure to the UV lights. In either case, USERS
SHOULD NOT CONTINUE TO WORK IN THE FACILITY IF THE
OVERHEAD UV LIGHTS ARE ON.

7. If the negative pressure alarm goes off (the overhead red lights start blinking),
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
leave everything in the hood and exit the BL-3, removing PPE in the anteroom as
usual. Work should not resume until the alarm stops.

8. Should these emergencies in the BL-3 take place, contact the BL-3 RA or
Director to notify them of the situation.

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 Research Assistant before carrying out this procedure. Depending on the item, either a fresh 10% bleach solution or a fresh 1:100 dilution of Amphyl can be used to wipe containers that are leaving BL-3. DOES will tell you to use 10% bleach. Amphyl (NOT Amephyll II spray) is EPA-approved for both MTB and HIV-1, and is therefore also approved by DOES.
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 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 leakproof 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 or a fresh 1:100 dilution of Amphyl 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.

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.

D. 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 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 doubled red biohazard bags. 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.
   b. Fill a plastic beaker approximately 10% with bleach. This is for small amounts of culture medium, infectious or not. All medium must be disposed off in 10% bleach.
   c. Fill a pipette trough approximately 1/3 full with bleach. 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. If the user has large amounts of waste, multiple troughs should be used as overfilling could create a hazardous situation.**
   d. If needed, use a vacuum flask filled 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 filter must be used between the flask and the vacuum line.

5. All SHARPS (i.e., glass slides, coverslips, needles) must be disposed in the approved red SHARPS container that has been placed inside of each hood. When the container becomes filled or is only partially full at the end of a Friday it must be securely closed and a piece of autoclave tape must be applied over the lid. The container must be placed in the autoclave when it is available, and sterilized for one hour. Before work may proceed, an empty SHARPS container must replace one that has been removed for sterilization.

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 (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 Amphyl II spray. 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 a disinfectant (Amphyl II spray) 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 must sit for 15-30 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. If you need to
leave the facility, you can label the container with “MTB”, your name, the
time and date and leave it for the BL-3 RA to discard. (If you're working
with virus, it only needs to soak in bleach for 2 minutes.)
c. After pouring the liquid waste into the sink, rinse the containers out with
water, place them in the carboy and completely cover them with a 1:100
dilution of Amphil. Mark the log sheet whenever you make fresh
solutions, add items to a solution made earlier that day or empty the
carboy. The items must soak for at least 10 minutes, and the RA will
empty the carboy at the end of the day.
d. All reusable plasticware used in the hood should be immersed completely
in the carboy containing a freshly-made solution of 1:100 Amphil
e. After soaking the reusable plasticware and containers should be
thoroughly rinsed with tap water and placed in the dish rack to dry. Pour
the used Amphil down the sink. A freshly-made solution of Amphil must
be made daily as necessary to effectively disinfect reusable plasticware.
Do not use an Amphil solution that was diluted more than 24 hours ago.
f. For pipette troughs that were used in MTB experiments:
make sure all items are completely submerged in 100% bleach. Tape the
lid to the trough with autoclave tape and write "MTB", the date, the time
and your name on the tape. Leave the trough in the hood under the UV
light. Troughs only need to soak for 15-30 minutes (2 minutes for virus),
so the RA will drain the bleach and autoclave everything later that day.
When autoclaving is finished, the RA will scrape the contents into the
sharps container next to the autoclave.
g. Wipe down the interior work surfaces of the hood with Amphil II spray
and place all wipe-up materials in the doubled red biohazard bags.
Remove the outer pair of gloves and put them in the doubled red
biohazard bags. Replace the outer pair of gloves. While keeping the
doubled red biohazard bags inside of the hood, loosely tape them closed
with autoclave tape, and leave them in the hood under UV light. The RA
will autoclave them at the end of the day.
h. The hood blower must be left "on" at all times.

11. All users are responsible for the general orderliness of the BL-3 facility. The RA
will do the autoclaving once a day, empty the carboy, 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.

E. Spills:

*Mycobacterium tuberculosis* (MTB)

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.
3. Pouring carefully, saturate the spill with bleach and let the bleach sit for at least 2 hours.
4. Turn "on" the overhead UV lights and post a sign on the entry door to the BL-3 area prohibiting entry. Note: the UV lights will automatically turn "off" when entry is made through this door.
5. Notify the BL-3 Research Assistant or the Director of the BL-3 facility.
6. After 2 hours, put on clean Personal Protection Equipment (PPE) to return to the spill.
7. 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).
8. Remove the solidified spill with a scoop (found in the spill kit), or with paper towels and dispose all of the waste inside of doubled biohazard bags.
9. 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.
10. Tie the biohazard bags loosely with autoclave tape, place the bags inside the autoclave and immediately sterilize them for one hour (cycle 4 on the autoclave touchpad).
11. An incident report must be written for any spill that occurs outside of a BL-3 lab hood.

Virus including HIV-1

1. In the event of a spill outside of a hood, dam off the spill with paper towels and post a warning sign at the entryway to the spill area to notify others.
2. Pouring carefully, saturate the spill with bleach and let the bleach sit for 30 minutes.
3. 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).
4. Remove the solidified spill with a scoop (found in the spill kit), or with paper towels and dispose all of the waste inside of doubled biohazard bags.
5. 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.
6. Tie the biohazard bags loosely with autoclave tape, place the bags inside the autoclave and immediately sterilize them for one hour (cycle 4 on the autoclave touchpad).
7. Notify the BL-3 Research Assistant or the Director of the BL-3 facility.
8. An incident report must be written for any spill that occurs outside of a BL-3 lab hood.

F. Exposure:
1. In the event of a potential exposure to a pathogen, first remove the contaminated garments/gloves as quickly as possible to reduce skin contamination.
2. Wash the exposed area gently with soap and warm water.
3. If mucous membranes are involved, wash the exposed area with copious amounts of water. An eyewash kit is available in the BL-3 facility.
4. Report the incident to the BL-3 RA, to your supervisor and to the University Health Service, located at 2145 Adelbert Road. During regular working hours, go directly to the University Health Service or call the needle-stick hotline (368-6635). Identify yourself as a BL-3 facility user. The University Health Service has a record for each user to keep track of his/her health status.
5. An incident report of the accident must be filled out. These forms are available at the University Health Service.
6. If exposure involves HIV, recommendations for treatment based on the severity of the exposure will be made according to Guidelines.
7. If the exposure involves MTB, repeat PPD skin testing will be required and the user will be referred to his/her own physician or to the University Health Service for recommendation on INH prophylaxis.
8. If the exposure involves UV radiation, immediately turn off the UV light as indicated and notify the BL-3 Director or RA so that appropriate action can be taken. Emergency treatment is not required in this situation provided appropriate PPE (as required for the BL-3) is worn.
9. Other than for immediate emergency response, no treatment should be offered by University faculty or staff at CWRU.

G. 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.

H. Safety Notes:
1. The overhead UV lights emit germicidal UV-C type radiation (short UV, 290-100 nm). Although it may lead to reddening of the eyes or conjunctivitis upon exposure, exposure does not lead to skin cancer or cataracts in humans.
2. UV-C is absorbed in the outer layers of skin and eyes so irritation produced upon exposure is superficial and, although burns may feel incapacitating at the time, the effects usually disappear within 24 hours without lasting effects.

3. 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.

4. 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. Both utilize HEPA filters. The primary means of sterilizing surfaces is the use of 10% bleach or AmphyII. UV light 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).

5. Disinfectants:
   a. AmphyII liquid (Active Ingredients: Benzyl-4-chlorphenol 5.0%; Phenylphenol 10.5%) is approved by the Environmental Protection Agency (EPA) as a disinfectant for Mycobacterium tuberculosis and HIV-1. It should be used at a dilution of 1:100 and made fresh daily. The BL-3 has confirmed that AmphyII at 1:100 is in fact tuberculocidal. Items and surfaces should soak in AmphyII for 10 minutes for it to be effective.
   b. AmphyII spray is ethanol-based and is also tuberculocidal according to the canister, but is not approved by the EPA. In its spray canister, it obviously doesn’t need to be diluted fresh daily, so it is much more convenient for daily use. AmphyII spray should be used for disinfecting within the BL-3, such as for items in the hoods. It is not approved for use for disinfecting items to be removed from the BL-3.
   c. Bleach is an approved disinfectant (by DOES) for items to be removed from the BL-3. A 10% solution must be made fresh daily. Bleach can corrode metals, such as the biohoods, so it is not ideal for such use.
III. CDC/NIH Guidelines for BL-3 Laboratories:

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets (BSC; "hood") or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment (PPE). The laboratory has special engineering and design features such as access zone, sealed penetrations, and directional airflow.

A. Standard Microbiological Practices:

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after handling infectious materials, after removing gloves, and when they leave the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield.
4. Mouth pipetting is prohibited.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of aerosols.
7. Work surfaces are decontaminated at least once a day and after any spill of viable material.
8. All cultures, stocks and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving.
9. An insect and rodent control program is in effect.

B. Special Practices:

1. Laboratory doors are kept closed when experiments are in progress.
2. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. Persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
3. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory.
4. When infectious materials or infected animals are present in the laboratory, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory access doors. The hazard warning sign identifies the agent, lists the
name and telephone number of the laboratory director or other responsible persons, and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators or other personal protective measures.

5. Laboratory personnel receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

6. Baseline serum samples are collected and stored for all laboratory and other at-risk personnel. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory.

7. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures.

8. Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural changes.

9. The laboratory director is responsible for ensuring that, before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques, and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in safe microbiological practices and techniques.

10. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative. Plasticware should be substituted for glassware whenever possible. Used disposable needles must not be bent, sheared, broken, recapped, removed from syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps.

11. All manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the laboratory. No work in open vessels is conducted on the open bench.

12. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during handling, processing, storage, transport, or shipping.

13. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes or other contamination with infectious materials. Spill procedures are developed and posted. Contaminated equipment must be decontaminated before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.
14. Spills of infectious materials are decontaminated, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material.

15. Spills and accidents which result in overt or potential exposures to infectious materials are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

16. All potentially contaminated waste materials from laboratories are decontaminated before disposal or reuse.

17. Animals and plants not related to the work being conducted are not permitted in the laboratory.

C. Safety Equipment (Primary Barriers):

1. Properly maintained BSCs are used for all manipulations of infectious materials.

2. Outside of a BSC, appropriate combinations of PPE are used (e.g., special protective clothing, masks, gloves, face protection or respirators), in combination with physical containment devices (e.g., centrifuge safety cups, sealed centrifuge rotors).

3. Respiratory protection is worn when aerosols cannot be safely contained.

4. Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits or coveralls must be worn in, and not worn outside, the laboratory. Reusable laboratory clothing is to be decontaminated before being laundered. Clothing is changed when overtly contaminated.

5. Gloves must be worn when hands may contact infectious materials and contaminated surfaces or equipment. Disposable gloves should be discarded when contaminated, and never washed for reuse.

D. Laboratory Facilities (Secondary Barriers):

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted. Passage through a series of two self-closing doors is the basic requirement for entry into the laboratory from access corridors. A clothes change room may be included in the passageway.

2. Each laboratory contains a sink for handwashing. The sink is hands-free or automatically operated and is located near the laboratory exit door.

3. The interior surfaces of walls, floors, and ceilings are resistant to disinfectants and chemicals so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontamination.

4. Benchtops are impervious to water and resistant to acids, alkalis, organic solvents, and chemical disinfectants.

5. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.

6. Windows in the laboratory are closed and sealed.

7. A method of decontaminating all laboratory waste is available, preferably within
the laboratory.

8. Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily-traveled laboratory areas.

9. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air from "clean" areas into the laboratory toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building, and is discharged to the outside, with filtration and other treatment optional. The outside exhaust must be dispersed away from occupied areas and air intakes. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper.

10. HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system. When Class III biological safety cabinets are used they should be directly connected to the exhaust system.

11. Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent. Filters must be replaced as needed.

12. An eyewash facility is readily available inside the laboratory.

13. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

14. The Biosafety Level 3 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified, at least annually, against these procedures as modified by operational experience.

15. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment, the site conditions, or other applicable federal, state, or local regulations.

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. Zahra Toossi is Director Core E and this core. The BL-3 Advisory Group includes Dr. David Sedwick, Dr. Richard Silver, Dr. David McDonald, Dr Toossi, and Mr. John Schaffer. The BL-3 Research Assistant is Jennifer Bongorno.

Appendix B: Current List of User Labs, Their Projects and Their Priority Scores

Arts Lab
- Priority score 1A for HIV ARV Resistance; NIH-funded
  Project code = 1A

- Priority score 1A for Sensitivity of HIV Isolates to RANTES analogues; NIH-funded
  Project code = 1A

Boom Lab
- Priority score 1A for TBRU funded project, MTB TLR signaling.
  Project code = 1A

Silver Lab
- Priority score 1A for NIH funded RO1 on contact mediated host resistance to TB
  Project code = 1A

Ramachandran Lab
- Priority score 1A for NIH funded R21 on Cell compartments of virulent MTB
  Project code = 1A

Canaday Lab
- Priority score 1A for NIH funded R21 APC in HIV/TB
  Project code = 1A

Nygen Lab
- Priority score 1A for R21 MTB virulence factors
  Project code = 1A

Toossi Lab
- Priority score 1A for NIH-funded RO1 on TB/HIV
  Project code = 1A
Appendix C: Current List of User Labs and Their Assigned Identification Colors

Arts - yellow     Canaday - red
BL-3 - white      Nguyen - blue
Boom - pink       Silver - green
Harding/Ramachandra - orange   Toossi - purple

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 BL-3 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 BL-3 Research Assistant will inform all BL-3 lab users as soon as possible.
2. The BL-3 Research Assistant will post a sign on the outside door of the facility informing of the shutdown.
3. The BL-3 Research Assistant 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 BL-3 Research Assistant 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, two pairs of latex gloves (one pair donned beneath the elastic sleeve band and the outer pair donned over the elastic sleeve band of the Tyvek coveralls), shoe covers, fitted N95 NIOSH-approved respirator, and protective eyewear.
6. The BL-3 Research Assistant 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 BL-3 Research Assistant 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 BL-3 Research Assistant must accompany the maintenance/repair personnel into the BL-3 lab.
8. The BL-3 Research Assistant 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 handsoap inside the locker room 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 biosafety level 3 by the Centers for Disease Control and Prevention (CDC). Pathogens that have been assigned to biosafety level 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 description of the procedure to be used for the disposal of all waste that is generated by the manipulation of the pathogen
   f. 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 BL-3 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 BL-3 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.