

**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. BSL-3 General Laboratory Practices	7
C. Entry/Exit Procedures	9
D. Materials Handling	10
E. Working in a Tissue Culture Hood	11
F. Cell Sorter	14
F. Emergency Procedures for Disruption of Negative Air Pressure	14
G. Spills	14
H.. Exposures	15
III. Remarks	16
IV. Safety Notes	16
V. CDC\NIH Guidelines for BSL-3 Laboratories	17
VI. Appendices	17
A. Current Directors and Members	17
B. Current List of User PIs	17
C. Current List of User Labs and Their Assigned Identification Colors	17
D. General Guidelines for Maintenance/Repair Procedures Performed within the BSL-3 Laboratory by Personnel Other than BSL-3 Lab Users.....	18
E. Procedure for Manipulating a New Pathogen within the Biosafety Level 3 Facility...	18

## **I. CWRU Biosafety Level 3 Organization, Equipment and Layout**

### **A. Organization:**

The Elizabeth A. Rich Biosafety Level 3 (BSL-3) Facility 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.

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

The BSL-3 Advisory Group meets quarterly or as needed to discuss the operations of the facility, any new projects, and any new or pertinent safety issues. Project approval or new pathogen approval is obtained by submitting a proposal to the BSL-3 RA who will take the request to the BSL-3 Director and Advisory Group. The BSL-3 Advisory Group also discusses operation of the BSL-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 BSL-3 facility are required to submit a summary of their progress upon request, or annually to the BSL-3 Advisory Group for the purpose of the BL3 progress report.

The responsibilities of the BSL-3 RA include:

- Teaching new investigators and technicians the standard operating procedures for performing their research in BSL-3 facility
- Maintaining records of BSL-3 usage for reporting and in order to process fees for usage.
- Enforcing biosafety rules and regulations.
- Managing certifications for usage and annual requirements which include respirator trainings, health screenings, and safety exams
- Attending to all aspects of safety issues/emergencies that have arisen from work in BSL-3.
- General maintenance of the BSL-3 facility and its equipment and maintaining supplies inventories.

The BSL-3 RA is the user's point of contact for concerns regarding the BSL-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 their concerns.

All activities in the BSL-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 BSL-3 laboratories and is provided for your information.

**B. Equipment and Layout:**

The BSL-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 two rooms used for biohazard work and an additional room for storage. Because the facility is for multiple users and its equipment is shared, cooperation and respect between users are imperatives. The following is a list of equipment maintained in the facility:

BSCs

Four, 6-ft  
One, Bio-bubble

Centrifuges

Two table top  
Two microcentrifuges

Incubators

Two CO<sub>2</sub>  
One roller incubator  
Three shakers

Microscopes

One inverted  
One light  
One light, with computer  
One StereoScope  
Colony counter system

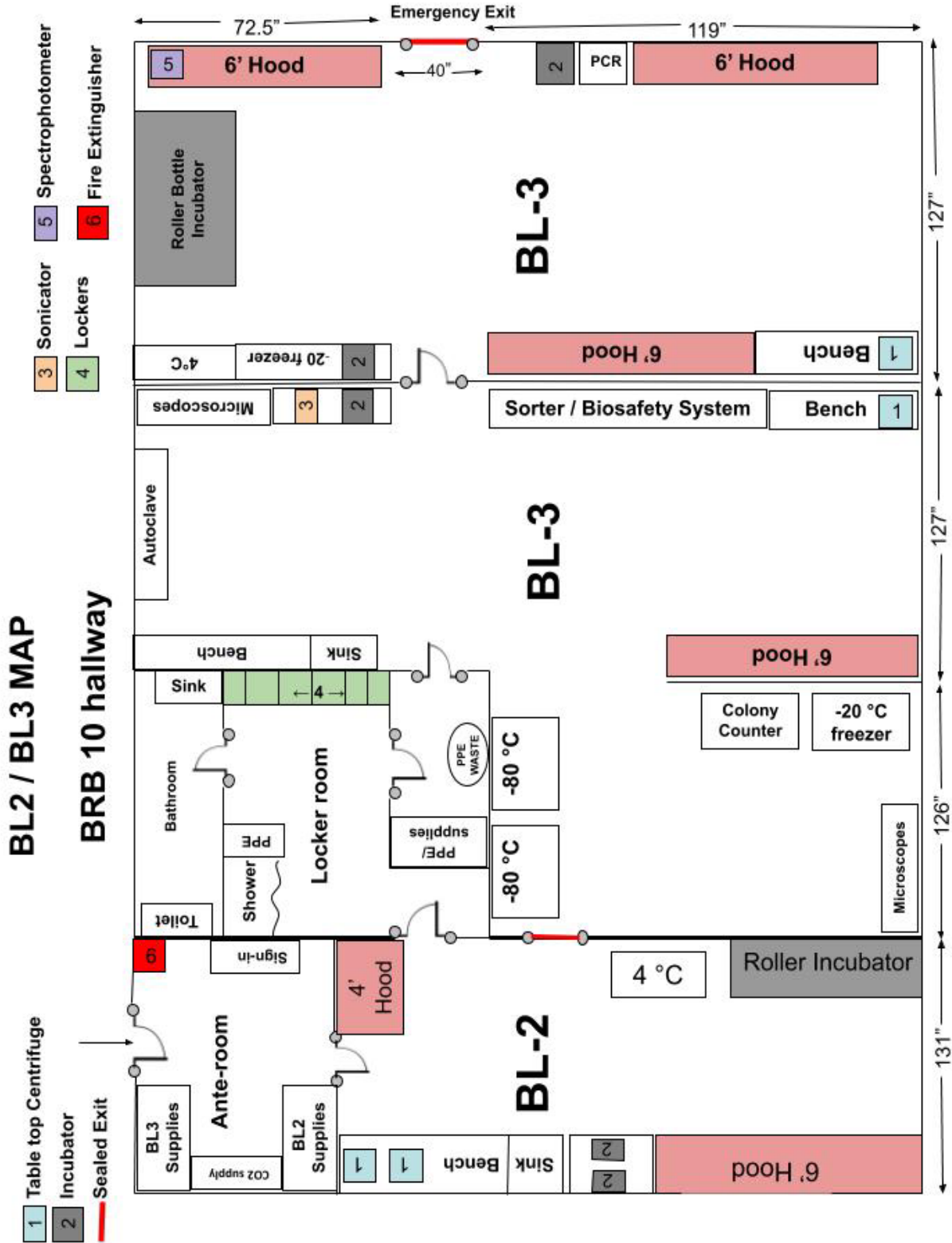
Refrigerators / freezers

One refrigerator  
Two -80 freezers  
Two small -20 freezers

Other

SONY Cell Sorter  
ELISA plate reader  
Sonicator  
Cell Density Meter  
Luminometer\*  
Thermocycler\*  
PCR machine  
Cell Density Meter

\* Not currently in the lab, however can be moved into the lab if and when needed.



## II. Procedures

### A. Requirements of Users: Certification and Project Approval

1. All users are required to be familiar with the contents of the BSL-3 Guidelines. Users initially must take and pass a written examination based upon the BSL-3 guidelines (including Parts I & II herein) before using the BSL-3 facility, and subsequently pass a yearly refresher exam.
2. Users must have a series of two baseline PPD's, or one quantiferon upon initial certification or if more than 18 months have elapsed since the last TB screening. Annual renewal of certification requires one PPD skin test or quantiferon test unless the user has previously tested positive. If there is a history of a positive PPD, a symptom questionnaire must be completed annually.
3. Covid vaccination requirements are regulated by the University and/or each PI. Covid vaccinations are strongly recommended for users working with any SARS CoV2 viruses.
4. Users must receive a tour of the facility from the BSL-3 RA, which includes an introduction to the rules and general operating procedures for the lab.
5. In addition to the initial tour mentioned in item 4, users must receive on-the-job training and supervision by the BSL-3 RA or an approved user before he or she is certified to work independently in the facility. It is assumed that all users have prior experience in handling tissues, cell cultures, and pathogenic organisms. The BSL-3 laboratory is not a training facility for these skills. This training will cover the specific safety protocols and procedures that are unique to working in the BSL-3 lab.
6. No rotation students or summer students will be certified to use the BSL-3.
7. Users must be annually trained and successfully fitted, by their employer (CWRU Environmental Health and Safety, VA, UH, etc.), for a NIOSH-approved N95 respirator. Respiratory training is also required in order to use a PAPR (Powered Air-Purifying Respirator). It is recommended that users are trained for and fitted to use both the PAPR and an N95.
8. Users (and P.I.s, if requested) must attend special BSL-3 laboratory meetings when called. These meetings will be held quarterly and can also be scheduled as needed.
9. P.I.s must have projects approved by the Advisory Group before work by their laboratory staff begins in the BSL-3 facility, and must submit progress reports yearly or upon request.
10. Each P.I. who wishes his/her lab group to use the BSL-3 facility may elect either to be "BSL-3 **qualified**" or "BSL-3 **non-qualified**". **Only** "BSL-3 qualified" P.I.s may enter and use the BSL-3 facility;
  - a. BSL-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 BSL-3 facility.
  - b. BSL-3 non-qualified

- i. the P.I. completes and signs a PI Agreement Form, indicating that he/she understands the rules of the lab and does not wish to become "BSL-3 qualified".
  - iii. the P.I. **may not** enter and use the BSL-3 facility.
11. P.I.s must sign an agreement to abide by and to help enforce the facility guidelines in order to begin using the facility..

## **B. BSL-3 General Laboratory Practices:**

1. 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 BSL-3 Guidelines is grounds for loss of BSL-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.
2. Users should report any conditions perceived to be unsafe to the BSL-3 RA. Any problem that cannot be resolved by the BSL-3 RA, will be addressed by the Core Director and the BSL-3 Advisory Group.
3. When entering the BSL-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 BSL-3 and is also used for billing purposes.
4. Open shoes or sandals are not permitted in the BSL-3 laboratory.
5. The following items are prohibited in the BSL-3 laboratory:
  - food
  - beverages
  - chewing gum
  - corrugated cardboard
  - lab-coat
  - purses
  - the use of any personal electronic and auxiliary devices such as cell phones, smart watches and headphones/earbuds.

*(Cell phones may be brought in under special circumstances and only with permission).*
6. Paper and pens may be brought into the BSL-3 laboratory, but cannot leave.
7. BSL-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.
8. The lab, refrigerators, and -80° C freezers all have 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.
9. 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 checked visually at least once per week. The BSL-3 RA conducts random spot checks of all incubators. If a culture goes more than a week without being checked, the user will be notified. If the user does nothing within the next 24 hours, the RA will issue a warning and has the right to discard the culture without further warning, if there is evidence of contamination. If a person has cultures discarded twice by the BSL-3 RA, the person will have his/her BSL-3 use suspended. Users who cannot fulfill this requirement may ask another certified user to do it for them.

10. To avoid the possibility of spreading 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 small biohazard bag, immediately seal the bag and spray the outer surface before placing it in a second bag which should also be sprayed. Plates with liquid media must be sealed and triple bagged. Spray and then secure the biohazard bag with autoclave tape and autoclave the contaminated material immediately. If a contaminated culture is found upon inspection by the RA, 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.
11. It is recommended that only filtered pipette tips be used in the facility.
12. It is recommended that fungizone be used in culture media whenever possible.
13. Only filter-top flasks may be used in the BSL-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.
14. The waste container of the ELISA platewasher should contain an EPA approved disinfectant and its fluids must be treated as biohazardous waste. Allow the fluid waste to remain in the disinfectant solution for at least ten minutes before washing the mixture down the sink with a copious amount of water. The waste container should be emptied and rinsed after each use.
15. Safety cups must be used on centrifuge rotors at all times. Tubes and plates that are used for balancing the centrifuge must be discarded or stored properly after each use. Plates or tubes with any liquid should never be left in common areas and will be discarded immediately even if they are labeled for balancing.
16. All manipulations of cultures must be done in a biosafety cabinet (hood).
17. 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.



18. Troughs with contents must be decontaminated prior to disposal. Allow trough contents to soak in 10 - 20% bleach solution for at least 10 minutes. Drain the bleach solution while flushing with running water, rinse and then autoclave the troughs with contents along with your biohazard waste using the LAB WASTE cycle.. The autoclaved contents must be emptied immediately into a sharps bin located beside the outer autoclave door to prevent a sharps hazard when they have cooled. Please try to thoroughly drain the bleach solution and then rinse the contents before autoclaving in order to prevent corrosion of the autoclave over time.
19. In the event that the autoclave is not functioning, the following procedures will be followed: If the autoclave can be fixed within a few days, waste generated inside the BSC will remain inside the BSC. This waste will be double bagged, sprayed and stored in one designated hood until the autoclave is fixed. Troughs with waste will soak for 20 minutes. Troughs will then be drained and rinsed, then sprayed and removed from the BSC by the RA for autoclaving. Solid waste from the troughs will be emptied into sharps boxes as usual. Waste generated outside of the BSCs can be bagged and stored in the BL3 until they can be autoclaved. In the event the repair time will exceed two weeks, all items must be decontaminated with 10% bleach before being placed into red biohazard bags. The bags will be sprayed and brought out of the BL3 to be autoclaved by the RA. Troughs will be handled as described above.

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

### **C. Entry/Exit Procedures: Donning and Doffing**

1. The hallway door of the BSL-3 facility is always locked. Each user of the BSL-3 must enter the facility by scanning his/her own CWRU identification card. 2. Prior to each entry into the BSL-3 lab, the user should sign into the logbook in the main anteroom.
2. Walk over the "Tacky Mat" so that particulate matter is removed from the bottom of your shoes.
3. In the locker room, change from your street clothes into scrubs, and hang your clothes in an assigned locker. Don a complete set of PPE (Personal Protective Equipment) . 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 BSL-3 ante-room and should be donned last. These will remain in the anteroom as they are reusable and must be sprayed/wiped 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 BSL-3 ante-room and are only used with an N95 (not needed with a PAPR)
4. When exiting, remove all PPE in the BSL-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. PAPRs must be sprayed and wiped with an EPA approved disinfectant upon removal. Used PPE must never be worn outside of the BL3 ante-room.
  5. 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 designated locker to be reused. Once these scrubs are dirty, place them in the “scrub laundry” bin. The Laundry bin will be emptied as needed. Only scrubs placed in the laundry bin will be taken for cleaning. The RA will not clean your lockers to collect dirty laundry.
  6. **All personnel must wash their hands in the locker room bathroom before leaving the facility.**
  7. Walk over the tacky mat to enter the main anteroom.
  8. Complete the Sign In/Out log to indicate the time leaving the facility.

#### **D. Materials Handling: Removing items from the BSL-3**

3. Equipment that must leave the BSL-3 facility must be ***COMPLETELY*** decontaminated with an EPA approved disinfectant and must follow the appropriate procedures. Check with the BSL-3 RA to confirm the proper procedure for removing your item from the lab.
4. Samples may be taken from the BSL-3 lab for further analysis with the permission of the BSL-3 RA or the BSL-3 Lab Director. Taking samples from the BSL-3 lab without permission is grounds for loss of BSL-3 lab use privileges. Once permission is granted, users do not need to obtain permission each time if they are removing the same material using the same procedure, however, the user

must complete the form from removing live or unfixed material from the lab EVERY TIME.

The following describes the procedure that must be utilized for the safe transfer of culture-derived material from the BSL-3 lab to the outside environment:

- a. The PI or user will consult with the RA or the BSL-3 Lab Director about the transfer and obtain his/her approval of the transfer procedure **before** it occurs. Once approval has been granted and every time after, the user must complete the form for removing live or unfixed material from the lab.
  - b. The primary container (i.e., vial, conical tube, bottle) holding the material must be secured (i.e., the cap (s) or top(s) must be tightened) to prevent leakage.
  - c. The primary container must be decontaminated by an EPA approved disinfectant then 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 must be secured (i.e., the cap(s) or top(s) must 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 sprayed and then wiped down with a fresh solution of \*10% bleach or LpH immediately prior to the container's removal from the BSL-3 lab.
  - e. The secondary container will not be opened outside of the BSL-3 facility prior to the container's arrival at a suitable storage facility (i.e., freezer) or biosafety cabinet, if the sample has been completely inactivated. If the sample is not inactivated, it can **ONLY** be removed in another BSL-3 and inside an approved BSC.  
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 BSL-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 BSL-3 facility.
  6. Paper must **never** be removed from the BSL-3 lab. You may use the laptop to email or transfer any data from the lab.

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

1. **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.

- b. Fill a pipette trough approximately 1/3 full with 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.**
2. If needed, use the vacuum aspirator collection system to aspirate fluid. The container must be prefilled with 100% bleach up to the 10 percent mark. The contents of the collection system must be allowed to sit for at least 20 minutes then flushed down the sink with at least twice the volume of water. If you need to use this system, please remember to reserve this biohood ahead of time. Alternatively, you may 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.
3. Change outer exam gloves frequently, discarding them inside the BSC, when working with infectious materials and when the gloves become contaminated, torn or punctured. Your inner gloves can only be removed in the lab if it becomes punctured or torn. If this should happen thoroughly wash your hands with soap and water before placing the gloves.
4. Do not clutter the hood because this interferes with proper airflow.
5. 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
5. Large amounts of 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 be sprayed immediately before removing it from the hood to be autoclaved. Small amounts can be disposed of in your trough along with your pipette tips.
6. In case of broken glass, 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 an EPA approved disinfectant solution depending on the pathogen that you are working with. For SARS related work: Use 70% ethanol. For Mtb: Use LpH or 10% bleach solution. 70% ethanol cannot be used to disinfect if you are working with Mtb.
  9. Any spill inside the hood must be immediately treated with an EPA approved disinfectant and wiped up with absorbent tissues. If any disinfectant solution such as bleach or LpH is spilled in the hood, it should be cleaned up immediately so it won't corrode the hood.
  10. Clean up when you're finished working in the hood:
    - a. For pipette troughs: Make sure all items are completely submerged in 10 - 20% bleach. Filled troughs must soak for AT LEAST 10 minutes. After 10 minutes, pour the disinfectant in the sink, flushing continuously with running water. Open the trough enough to collect enough fresh water to rinse away as much of the bleach solution as possible. Bleach in the autoclave will cause corrosion over time. Autoclave the trough and contents with your biohazard waste using the LAB WASTE cycle. Once the cycle is complete, remove the trough and dump the contents in the red sharps bin. Return the trough to the lab. Dispose of the SHARPS bin by autoclaving once it is full.
    - b. The beaker and flask containing bleach solution with waste must sit for AT LEAST 10 minutes before discarding the liquid waste down the sink. All liquid waste discarded in this manner must be flushed with at least double the volume of water. 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 EPA approved disinfectant solution. The items must soak for at least 10 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.
    - g. Wipe down the interior work surfaces of the hood with an EPA approved disinfectant solution depending on the pathogen you are working with, and place all wipe-up materials in the red biohazard bag inside the hood. To prevent corrosion from bleach or LpH, PLEASE WAIT AT LEAST 10 MINUTES before doing a final wipe down with 70% ethanol provided in spray containers. 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 disinfectant solution before carefully removing and then placing it in a second biohazard bag. Autoclave using the LAB WASTE cycle.
    - h. The hood blower must be left "ON" at all times.

**F. Cell Sorter**

1. All users must be trained BEFORE using the sorter. Please contact the BL3 RA to arrange training.
2. Users must use face shields, or PAPR, not safety goggles, while using the sorter
3. The Sorter Containment System or biobubble is a Biosafety container and must be treated as such. No **OPEN** samples should be removed from this biobubble.

**G. Emergency Procedures for Disruption of Negative Air Pressure**

If the air balance has been disturbed, the alarm will activate on the biosafety cabinets 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 BSL-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. If the negative pressure is restored and the alarm stops within 60 seconds, the user may return to his/her work. If the alarm remains active, the user must spray their coveralls with 10% bleach solution or LpH and exit the lab immediately. Upon exiting the lab, a sign must be posted to prohibit other users from entering the facility.

Contact the BSL-3 RA or Director to notify them of the situation. Wait for instructions before re-entering the lab.

**G. Spills:**

1. In the event of an MTB spill outside of a hood, notify the other workers inside the BSL-3 lab. **Everybody must exit the facility.** Avoid inhaling airborne material and spray coveralls with 10% bleach or LpH before exiting the lab. Remove your PPE and exit to the locker room to wash your hands and don fresh PPE.
2. After 30 minutes, re-enter the lab to clean up the spill. Everything needed for cleanup is in an EMERGENCY SPILL KIT. Familiarize yourself with its location.
3. Contain the spill with absorbent paper towels or disposable pads. Carefully add 10% bleach to the spill. Avoid creating aerosols when pouring the disinfectant.
4. Leave the room for 30 minutes to allow bleach to inactivate the material.
5. Return after 30 minutes to finish the cleanup. Pick up any broken glass or sharps with the tongs or forceps provided and discard them in the sharps container. Decontaminate the tongs/forceps by allowing at least 20 minutes of contact time with 10% bleach before replacing it in the kit. Clean the area with paper towels and collect all contaminated materials into a biohazard bag.
6. Spray and wipe the spill area with 10% bleach or LpH solution.
7. Double bag all waste, spraying the outer surface of both bags and autoclave immediately.

8. Spray your tyvek suit with disinfectant before exiting the lab to don clean PPE.
9. Report the spill to the lab RA and YOUR PI.
10. Complete an incident report if you believe you have been personally exposed (eyes, skin or mucous membranes) or if advised to do so.

## H. 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:

### Exposure to eyes, skin or any mucous membranes:

- . Immediately remove goggles or suit and wash the involved area with copious amounts of water. There is an eyewash station located by the sink in the BSL-3. Leave the contaminated suit inside the lab and proceed to the **ante-room**. Fresh scrubs are located in the EMERGENCY BOX in the ante room. Leave the dirty or contaminated scrubs in the laundry bag in the anteroom to be autoclaved. PAPRs must be completely wiped down with disinfectant solution. Enter the locker room to don fresh PPE before returning to clean the spill. Upon exiting the lab the user may shower before returning to street clothes. Notify the lab RA and YOUR PI and complete an incident report.

### Exposure to tyvek suit:

Remove suit immediately, leaving it inside the BSL-3 and then proceed immediately to the anteroom. Change into clean scrubs located in the anteroom and leave dirty or contaminated scrubs in the autoclave bag in the ante room. Proceed to the locker room in the clean scrubs, don fresh PPE and return to the lab to clean up the spill. Spray and discard the contaminated PPE in double biohazard bags and autoclave immediately using the LAB WASTE cycle

### Exposure to any clothing other than tyvek suit, ie. scrubs:

In the rare event that scrubs become contaminated, ask all other users to exit the lab immediately. Leaving the respirator and goggles/face-shield in place, remove contaminated suit and scrubs as quickly as possible. Use a pair of scissors to remove the top to avoid pulling it over your face. Proceed to the anteroom to put on clean scrubs located in the emergency box. Spray the ante-room with a light mist of LpH and enter the locker room into fresh scrubs and PPE. Return to the lab to clean up any spills. Spray and discard the contaminated PPE in a doubled biohazard bag. Upon exiting the lab the user may shower before returning to street clothes.

Notify the lab RA and YOUR PI and complete an incident report.

Each exposure will be handled on a case by case basis depending on the type of exposure

**III. Remarks:**

The BSL-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 BSL-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.

**IV. 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 BSL-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 and LpH can corrode metals, such as the biohoods, so it is recommended that after disinfecting with bleach, these be wiped with 70% ethanol after waiting 10 minutes.**

**Note: 70% ethanol is not an approved disinfectant for Mtb and therefore cannot be used except inside the BSC while working with SARS CoV2. Outside of the BSC ALL items must be decontaminated with an EPA approved disinfectant listed above.**



**V. Link to the current version of the CDC/NIH Guidelines:**

**Biosafety in Microbiological and Biomedical Laboratories, 6th Edition, June 2020**  
[https://www.cdc.gov/labs/pdf/SF\\_19\\_308133-A\\_BMBL6\\_00-BOOK-WEB-final-3.pdf](https://www.cdc.gov/labs/pdf/SF_19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf)

**VII. Appendices****Appendix A: Current Directors and Members**

The BSL-3 laboratory is a core facility of Case Western Reserve University School of Medicine. Dr. Henry Boom is the Lab Director. The BSL-3 Advisory Group includes the lab director, Dr. Henry Boom, a supervisor from EHS, Andrew Young, and three representative PIs, Dr. Richard Silver, Dr Anna Bruchez, and Dr. David Canaday. The BSL-3 Research Assistant is Sophia Masters Onwuzulike.

**Appendix B: Current List of User PIs**

Henry Boom

Stephen Carpenter

Anna Bruchez

Richard Silver

David Canaday

Richard Bonomo

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

1. All BSL-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 BSL-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 BSL-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 BSL-3 lab must don personal protective equipment (PPE) that is at least as protective as that being used by the BSL-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 or a PAPR.
6. The RA must encourage the maintenance/repair personnel to take into the BSL-3 lab the minimum amount of tools and instruments he/she will need to perform the required task. Before entering the BSL-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 BSL-3 lab.
7. The RA must accompany the maintenance/repair personnel into the BSL-3 lab.
8. The RA is responsible for supervising the safe and proper egress from the BSL-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 BSL-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 D: Procedure for Manipulating a New Pathogen within the Biosafety Level 3 Facility**

The Biosafety Level 3 (BSL-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 BSL-3 Facility.

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

1. Submit a written protocol proposal for the approval of the BSL-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 BSL-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 BSL-3 Advisory Group, the BSL-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 BSL-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 BSL-3 Facility will not be allowed to proceed before all of the above actions have been successfully completed.**