

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

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 CO₂
One roller incubator
Three shakers

Microscopes

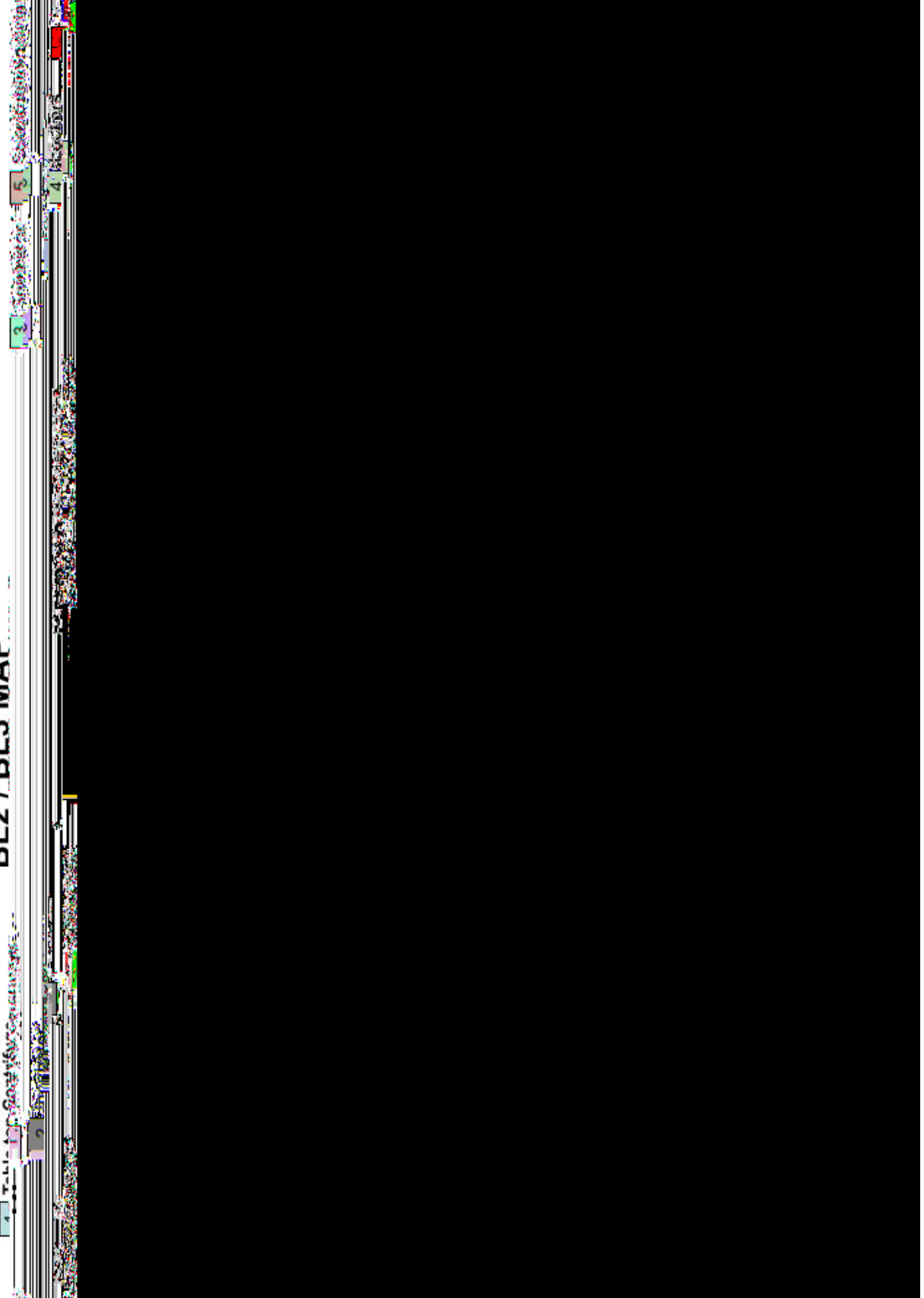
One inverted

Refrigerators / freezers

One

Other

BL2 / BL3 MAP



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.

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

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.

- 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

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 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 air flow 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 air flow 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.

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

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

VII. Appendices

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.

Henry Boom

Stephen Carpenter

Anna Bruchez

Richard Silver

David Canaday

Richard Bonomo

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

