

BL2+ WORK PRACTICES

Biosafety Level 2 Plus (BL2+) is the designation utilized for those biohazard experiments that require practices that are more stringent than standard BL2 procedures. Generally, BL3 practices are mandated in a space designed for BL2 work. It is preferred that the BL2 laboratory be self-contained, that is, all equipment required for the experiment should be located within the lab. A sign is posted on the door while BL2+ work is in progress and access is restricted to those involved in the experiment. When work is completed, and equipment has been decontaminated, the sign is removed and the lab returns to standard BL2 or BL1 usage.

BL3 practices require that all work be conducted under physical containment. Therefore, all manipulations with BL2+ material are conducted within a Class II biological safety cabinet and secondary containment is utilized for centrifugation and other potential aerosol generating procedures. The following notes further describe the requirements for work at BL2+.

Personal Protective Equipment (PPE)

- Dedicate PPE for the experiment. PPE worn for BL2+ work should not be worn in other areas. Remove before leaving the laboratory.
- Wear a lab coat or solid-front gown, preferably with a knit or grip cuff.
- Double glove for all work within the biological safety cabinet (BSC). Remove the outer pair before exiting the BSC, and don a new pair each time you reenter the BSC.
- Ensure that your gloves extend over the sleeve of your lab coat. An opening at the wrist will allow aerosols generated within the BSC to contaminate your wrist and forearm, extending handwashing to your elbow.
- Sleeve covers can be worn to ensure coverage of the wrist and will also minimize contamination of the sleeves of your lab coat.
- Face Protection (mask and eyewear can also be worn and will protect mucous membranes from exposure in the event a spill outside the BSC during transfer of material to and from the incubator. It will also help to prevent you from touching your eyes, nose and mouth when working within the BSC.
- Remove PPE before leaving the laboratory. Placing a hook within the BL2+ area will facilitate this requirement. Remove your outer gloves first, then your lab coat or gown, followed by the inner gloves. Take your face protection off last. Don't touch your face with gloved hands. Remove gloves and other clothing aseptically, from the inside out, and avoid touching the contaminated outer side of the glove.
- Decontaminate reusable PPE as soon as feasible after it has been contaminated. Small areas can be spot treated with a suitable disinfectant, such as 1-10% household bleach. Lab coats can also be autoclaved, or sent to a laundry facility equipped to handle biohazardous PPE. Disposable PPE can be placed within a biohazard bag, treated and discarded as biomedical waste.
- Wash your hands with soap and water after removing PPE and before leaving the laboratory.

Work Practices In the Biological Safety Cabinet (BSC)

- Perform all work within a BSC. This includes discarding waste within the BSC. Moving your hands in and out of the BSC will disrupt the protective air curtain at the front access opening.
- Place all items required for the experiment within the BSC before starting work.
- Wipe items down with disinfectant prior to placement within the BSC.
- Segregate clean areas from contaminated areas within the BSC (by at least 12-14”).
- Keep the front and rear grilles clear when working within the BSC. Avoid blocking the rear grille. Don't store items on top of the BSC. Remind fellow researchers to minimize traffic and work behind the operator, as this may interfere with cabinet airflow. Depending on the location of the BSC within the room, opening and closing the room door can significantly interfere with BSC airflow.
- Avoid the use of a flame within the BSC. In addition to presenting a fire hazard, an open flame can disrupt airflow and possibly damage the paper filter located above the work surface. If the use of flame is absolutely necessary, use a burner with a pilot light that provides a flame only when depressed and releases after contact. Never leave an open flame (burner or pilot light) unattended in your BSC.
- Store tissue culture flasks in the incubator within small secondary trays to help minimize contamination. Trays will also facilitate transfer to and from the BSC.
- Keep your hands away from your face (face protection helps to minimize the potential for this route of exposure).
- Avoid the use of glass Pasteur pipettes or needles and syringes. Substitute plastic for glass whenever feasible. Alternatives to glass Pasteur pipettes include: plastic pipettes, plastic transfer pipettes, plastic gel loading pipette tips and pipette tip extendors, aspirators, and flexible plastic aspiration pipettes. Some researchers will either score and break the end off of a 1 ml or 5 ml plastic pipette or remove the wool plug and use for aspirating cultures.
- If the use of sharps cannot be avoided, maintain a sharps container in the immediate vicinity of use. Discard intact needles and syringes immediately after use. Use a one-handed disposal method (keep a hand behind your back or by your side, don't place on or near the opening of the sharps container). Never recap, bend, break or otherwise manipulate sharps by hand. If you must remove the needle from the syringe, use the small opening on the top of the needlebox for this purpose. Forceps, tweezers, or small pliers may also be utilized.
- Protect the house vacuum system or pump from contamination by installing a trap and filter system. Use a primary collection flask containing disinfectant, followed by an overflow flask, which leads through a HEPA or hydrophobic filter. Please see the Vacuum System Protection Handout. Vacuum filters are available in the Medical School Stockroom, SHM IE-7.
- Collect all waste within the BSC. Smaller biohazard waste bags may be utilized along with beakers or shallow trays containing disinfectant for the collection and disinfection of pipettes and other contaminated items. Waste can also be collected within the BSC in the following manner.

Horizontal collection: Horizontal trays containing disinfectant allow total immersion of pipettes.

Vertical collection: Beakers containing disinfectant can be used if disinfectant is drawn up inside the pipette and allowed to run down the interior wall upon disposal into the beaker.

Bags: Bags have the potential for creating aerosols when moved. At BL2+, seal autoclave bags within the cabinet and place within a second bag. Carefully add water to the primary bag before sealing (25 ml for smaller bags, 200 ml for larger bags). The addition of water will help to generate steam within the bag during the autoclave cycle.

- Wipe items down with disinfectant prior to removal from the BSC.
- Wipe down BSC with disinfectant after use (work surface, grilles, sides, back and inside front view screen).
- Decontaminate liquid waste with household bleach diluted 10% against the volume of the waste. Allow at least a 30 minute contact time for full decontamination.
- Transport waste to autoclave in a leakproof container.

Centrifugation

- ❖ Use sealed rotors or safety buckets as secondary containment for centrifugation.
- ❖ Load and unload the rotor or safety buckets within the BSC.
- ❖ Don't overfill primary containers, limit to $< \frac{3}{4}$ full. Wipe exterior of tube with disinfectant before loading.
- ❖ Seal rotor or bucket and wipe down with disinfectant, remove outer gloves, and transport to the centrifuge.
- ❖ Post a sign on centrifuge that includes the biohazard symbol, name of the agent with Biosafety Level, and your name.
- ❖ Wait 2-5 minutes after the run to allow aerosols to settle in the event of a spill. Transport sealed rotor or safety bucket to cabinet to complete your experiment. Don new pair of outer gloves.
- ❖ Decontaminate the rotor or safety bucket by spraying with 70% ethanol and allowing to air dry. Wipe the throw line within the centrifuge with disinfectant and remove your biohazard sign. In the event of a spill during centrifugation, follow the spill response procedures outlined in the Biosafety Spill Response Guide.
- ❖ Avoid the use of microfuge, which is difficult to contain. If you cannot avoid using a microfuge, use a model that has built in secondary containment (a sealed rotor) along with microfuge tubes equipped with a O-ring seal. You can also operate your microfuge in the rear of your BSC (don't perform any work within the BSC while the microfuge is in operation, and wait 2-5 minutes after the run before opening the microfuge).

Labels

- ◆ Post a biohazard sign at the entry to the BL2+ laboratory.
- ◆ Ensure that any specific entry requirements (vaccination), the name of the agent, the Biosafety Level, and the name of an emergency contact person is posted on either the sign or the Laboratory Information Card.
- ◆ Place the BL2 wall notice (not a door sign) inside your laboratory to remind researchers of the core safety practices.
- ◆ Label equipment housing the agent (incubators, freezers) with the universal biohazard symbol and agent name.

Transport of Biohazards

On Campus Transport (between labs or buildings):

- ✓ Must have two leakproof containers, including the following:
 - a sealed primary container
 - a sealed secondary container
 - absorbent (paper towels) between the primary and secondary containers suitable for the volume transported
 - a biohazard sticker on outside of the secondary container with agent name, lab address and phone number
- ✓ Utilize plastic containers whenever feasible, avoid glass.
- ✓ Sealed plastic (not glass) primary vials can be transported within sealed, labeled plastic bags.
- ✓ If glass primary containers must be used, place containers within a sealed rigid plastic container with absorbent and padding to cushion vials during transport.

Handwashing

- ❑ Wash hands whenever PPE is removed and before leaving the laboratory.
- ❑ Wash with soap and warm water for at least 15 seconds. Since the contact time of most soaps is quite extensive for actual decontamination, mechanical friction from scrubbing and water dilution are essential for complete cleaning.
- ❑ No glove is 100% leakproof.
- ❑ Never wet or handwash your gloves with water or disinfectant, as this will encourage wicking and increase permeability of the protective barrier.

Spills and Exposure Incidents

- All researchers must be familiar with the applicable exposure response procedures before initiating their experiments.
- Review the attached Biosafety Spill and Incident Response Guide before starting work.