

Biosafety Level 2

Work Practices

Adopted from
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HHS

STANDARD MICROBIOLOGICAL PROCEDURES - BSL 2

1. Access to the lab is restricted at the discretion of the laboratory director when experiments are in progress.

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General work practices for BL2+ Laboratories

Waste Overview

	NON- HAZARDOUS SOLID WASTE	INFECTIOUS SOLID WASTE	SHARPS	INFECTIOUS LIQUID WASTE
EXAMPLES OF WASTE	Pipets, plasticware, paper, glass (unbroken), gloves, etc. which have not been in contact with infectious material.	Pipet tips, plasticware, paper, glass (unbroken), gloves, etc. which have, or MAY have, come in contact with blood.	ALL broken glass, Pasteur pipets,	

<p>HOW WASTE IS DISPOSED OF</p>	<p>Close securely before putting into double-lined (red bag). Waste baskets with _____ will be emptied by Environmental Services staff each evening when labs are cleaned.</p>	<p>Waste will be autoclaved and disposed of as regulated medical waste.</p>	<p>Call the Service Response Center at XXX to let Biosystems know they have to do a pickup at Higgins.</p>	<p>Liquid waste: Sink dispose after bleach (let sit for at least 20min in bleach after infectious material is added). Flow cytometry tubes: When full, double red bag and place in a burn box for disposal without auto-claving.</p>
<p>SPECIAL INSTRUCTIONS</p>	<p>Serum bottles need to be disposed as medical waste (burn box).</p>	<p>Minimize the amount of liquid you put in these bags.</p>	<p>No bleach or other liquid in sharps containers.</p>	

Biosafety Procedure 1

Disposal of solid waste

DISPOSAL OF SOLID INFECTIOUS WASTE

Examples of waste:

Pipet tips, plasticware, paper, unbroken glass, gloves, etc. which MAY have come in contact with blood, cultured cells, or supernatants of cultured cells of a human or monkey origin, blue gowns.

How handled:

- Line a plastic bucket with an **orange autoclave bag**. Dispose of solid waste in this bucket. When full (or at the end of the day) close the bag securely with the rubber bands provided with the bags. Waste must not overflow the top of the bucket. Autoclave the orange bags.
- Place gloves

Biosafety Procedure 2

Disposal of sharps

Generally avoid using sharps in the BL2 tissue culture lab.

Examples of waste:

All broken or chipped glassware, pasture pipets, needles, razor blades, or anything else that could potentially puncture.

How handled:

Place in a _____ . Do not overfill the sharps containers. Replace them when they are " full. If alternatively, you are using a _____ for biohazardous sharps, make sure that the lid is still closable when filled.

How disposed of:

- For the _____ sharp container, close the lid of the filled sharps container and place it in a plastic bucket lined with an orange autoclave bag. Close the bag securely with the rubber bands provided with the bags. Autoclave and discard as regulated medical waste.

Special note:

Unbroken glass bottles or flasks are not considered sharps. They are collected in empty cardboard boxes. Once empty glass bottles have been collected, put the cardboard box in the hallway recognizable for the cleaning personnel that these are empty glass bottles for disposal.

Biosafety Procedure 4

Decontamination of biohazardous waste from BL2+ Laboratories

Waste affected:

All waste containing or suspecting to contain etiologic agents infectious to humans.

- # Any human or monkey blood (exposure risk examples: Hepatitis B or C, SIV, HIV, SHIV, and other bloodborne pathogens)
- # Cultured cells or supernatants from cultured cells, known or suspected to contain HIV, SIV, or other bloodborne pathogens.

Procedure:

1. All waste will be placed in autoclavable orange biohazard bags and the bags contained within a hard plastic bucket. This bag will be closed with the rubber band provided with the bags (or tape). Waste will not overflow the top of the bucket.
2. Autoclave (250°F) should be closed tightly. Set it for 60 minutes sterilizing time and 30 minutes drying time in the “Dry cycle”. Press start button. Make sure the pressure in the chamber rises to 18-20 mm Hg, and stays there for 60 minutes. Pressure should fall to 0 before pressing off button and opening door. Crack the door for 10 minutes.

After decontamination, bags of waste will be placed in a double-lined (red bag), in a 4 cu. ft. cardboard box. When full, the lining bags will be individually sealed with tape, the box taped closed, and then placed in the hallway for pick up by Environmental Services. See Appendix C Boston College Biohazard Manual for more detailed information.

Biosafety Procedure 5

Receiving blood samples of Human or non-Human Primate origin

1. John O'Grady (Higgins operations 552-6778) or Brad Garman (Higgins operation assistant 552-0058) will be informed when we are expecting a shipment (by FedEx for national shipments or a courier service for local shipments).
2. Blood specimens will be delivered to Receiving on the first level.
3. Blood samples, contained in a biohazardous shipping container (see Appendix A), will be taken to the BL2 cell culture laboratory (457) and will remain locked until the experiment begins.
4. Within the clean area, the inner plastic container will be removed from the cardboard box. The cardboard box remains in the "clean" area.
5. The plastic container will be moved to a biosafety cabinet where it will be opened and blood tubes inspected for leakage or breakage. If all are intact, specimens will be distributed to investigators. If any leakage has occurred, it will be treated as a biohazardous spill and the lab director or biosafety officer notified before proceeding.
6. Assuming no leakage, the plastic container will be decontaminated with Cold Spore or similar disinfectant. Plastic container will then be washed with other glassware.
7. After it is washed the plastic inner container will be returned to the cardboard outer container and the package reused.
8. Shipping containers will be discarded when inner shipping container becomes contaminated with blood or when the outer box becomes wet, contaminated, or its integrity otherwise compromised.

Biosafety Procedure 6

Action to take in case of accidental spill of biohazardous material

1. Notify others in the area that a hazardous spill has occurred. Get help if needed. Attend to any injuries first
2. If necessary, evacuate the room for 20-30 minutes so that any aerosol generated will settle and not be inhaled.
3. **CONTAIN** and **DISINFECT**:"
Use the spill kit located in each lab.
 - SPILL KIT contents:**
 - concentrated bleach solution
 - heavy (utility)gloves and regular (medical examination) gloves
 - absorbent materials for liquids
 - paper towels
 - forceps, tweezers, or other mechanicals for handling sharps
 - small biohazard sharps container
 - biohazard bag for disposal
 - shoe covers
 - face protection (goggles and face mask)
 - (- possibly solid front gown)
 - (- possibly full face shield)
 - A. Small spills:
Place paper towels over the spill to absorb it. Pour bleach on the paper towel and let it sit for at least 20 minutes before picking up the paper towel.
 - B. Large spills:
Block off spill with paper towel to keep it from spreading. Carefully pour bleach onto the spill, let it sit, then pick up paper towels.
4. Use tongs to pick up sharps or broken glass. Never use your hands.
5. Dispose of all clean up materials as hazardous waste.
6. Inform laboratory biosafety officer that spill occurred and how it was cleaned up.
- 7.

Biosafety Procedure 6A

Emergency Procedure for BL2+ Sorting Room

Follow all instruction that are outlined in Biosafety Procedure 6 (Action to Take in Case of Biohazardous Spill) and 11 (BL2+ Cell Sorting) to ensure your safety and that of others around you while containing the spill. If you have been exposed in the course of the accident, refer to Biosafety Procedure 8 (Emergency Procedures for Blood/Biohazard Exposure).

1. Immediately shut down the instrument to prevent the further spread of the spill/contamination.
2. Exit the room, follow the instructions provided in Biosafety Procedure 10 for removal of PPE, and notify all others in the area. Get help.
3. **DO NOT ENTER THE SORTER ROOM FOR AT LEAST 30 MINUTES AFTER THE SPILL.** This will allow most of the aerosols to settle.
4. **YOU MUST WEAR THE ENTIRE BIOSAFETY SUIT TO DECONTAMINATE THE ROOM.** It is advisable that there be at least one other person in the sorter room to help with the decontamination. Do not try to handle all of this on your own.
5. All porous surfaces that cannot be easily disinfected (i.e., ceiling tiles, pipe insulation etc.) must be removed, soaked in bleach, secured in a large orange autoclave bag and placed on a cart in one of the tissue cultures labs designated for items to be autoclaved.
6. All other surfaces must be decontaminated according to Biosafety Procedure 10. This includes the floors, walls, all furniture, the instruments and cabinets.

If you are unable to handle clean up yourself, call EH&S (Sunil 2-0363).

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completed and that no one should enter the sorter room.

F. **DO NOT EMPTY THE WASTE CONTAINER IMMEDIATELY AFTER SORTING.**

6. Removal of Personal Protective Equipment:
 - A. Remove the outer pair of gloves before exiting the sorter room and discard in a biohazard bucket that is lined with an orange autoclave bag.
 - B. Remove the blue gown, boot covers, hood, coverall and gloves and place them in the biohazard box that is lined with a large orange autoclave bag. This **must** be done outside of the sorter room.
 - C. Wipe down the helmet with gauze soaked in 70% ethanol.

7. Decontamination of the Sorter Room 12 Hours Post Sort:
 - A. Enter the room wearing a blue gown and gloves (standard BL2 procedures).
 - B. Spray down all surfaces with 70% ethanol then wipe the surfaces using gauze or paper towels. Discard the gauze/paper towels in a biohazard bucket lined with a small orange autoclave bag. Secure the bag closed using a rubber band.
 - C. Discard any remaining sample tubes in a sharps container.
 - D. Discard and replace the biohazard filter. Place the "dirty" filter in a biohazard bucket lined with a small orange autoclave bag.
 - E. Secure closed any remaining orange, autoclave bags using a rubber band.
 - F. Bring the biohazard buckets to one of the tissue culture labs and place it on a cart that is designated for items to be autoclaved.

THE WASTE CONTAINER MAY NOW BE EMPTIED INTO A SINK THAT IS DESIGNATED FOR LIQUID WASTE.



Biosafety Procedure 11

Centrifuge usage

Tubes with human or rhesus blood, serum or other bodily fluids must only be opened in the biosafety cabinet (Higgins 457).

1. Bring the centrifuge adaptors, buckets and sealing caps to the biosafety cabinet. Put your tubes in the centrifuge buckets with adaptors to spin, attach the sealing cap, and only then remove the entire sealed bucket from the biosafety cabinet into the centrifuge.
2. After the spin is completed, remove the entire sealed bucket from the centrifuge into the biosafety cabinet and then remove the sealing cap and samples.
3. Inspect for spills in the buckets (if there was leakage or a spill in the centrifuge buckets, refer to Biosafety Procedure 6 for decontamination.)
4. Before you remove the buckets from the hood, reattach the sealing caps.