

IBC
APPENDIX C
SAFE USE OF A BIOSAFETY CABINET
SOP

Purpose

To provide guidance on the safe operation of biological safety cabinets (BSCs) and ensure proper containment of biohazards.

Biological safety cabinets shall be used:

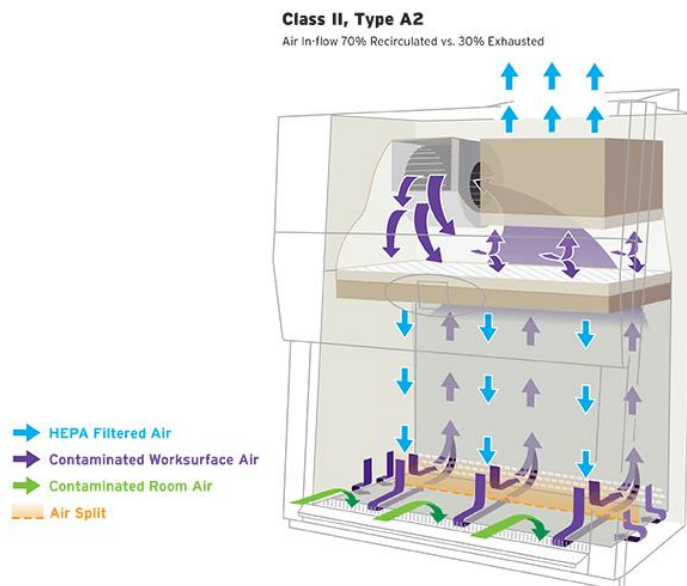
- To protect lab workers from accidental exposures and laboratory acquired infections while handling biohazardous materials at BSL-2 (or BL-2).
- To provide the clean environment necessary for propagation of cell cultures.

Basic Safety Guidelines

Users of BSCs shall follow the procedures described below.

1. All operators shall receive training in the safe operation of the BSC prior to use. Training may be delegated to a qualified individual, however it remains the responsibility of the Principal Investigator (PI) to ensure that lab personnel are appropriately trained.
2. Wear the required PPE, including barrier lab coat when working with human-derived materials, gloves, eye/face protection to protect you and your samples from contamination.
3. Ensure the work area inside the BSC is unobstructed. Place items to be used in the experiment adjacent to the side walls to ensure unobstructed airflow.
4. Keep sashes as low as possible when working in the BSC.
5. Keep sashes fully closed when the BSC is not in use.

Most of the BSCs at URI are Class II, Type A2. The airflow characteristics of this type of BSC are illustrated in the graphic below.



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For detailed information on the various types of biosafety cabinets see Appendix A, Bio-safety Cabinets, in *Biosafety in Microbiological and Biomedical Laboratories*, 6th Ed., p. 367.

https://www.cdc.gov/labs/pdf/SF_19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

It describes airflow patterns of the different types of BSCs. You can find the type of your BSC on the metal plate that includes the model and serial number.

Become familiar with the airflow pattern of your BSC. It will help you understand what makes the BSC a safe work environment. It will also help you see how easy it is to disturb the air curtain and create an unsafe work environment.

For additional information on the safe use of biosafety cabinets see the following videos on the NuAire website. NuAire is a major manufacturer of biosafety cabinets and the manufacturer of most of the University's BSCs.

- Proper Preparation - The 4 steps to take before you begin work in your biosafety cabinet <https://www.nuaire.com/resources/biosafety-cabinet-4-steps-before-work-video>
- Working in Your Biosafety Cabinet <https://www.nuaire.com/resources/working-in-your-biosafety-cabinet-video>

STANDARD OPERATING PROCEDURES

Preparing for Work within a Class II BSC

1. Wear a clean lab coat to avoid introducing contamination into the BSC.
2. Have a written checklist of materials needed for each activity.
3. Disinfect the work area before use. Wipe down the work surface, interior walls (except the supply filter diffuser), and inside of the window with 70% Ethanol or a suitable disinfectant. Several applications of 70% Ethanol may be necessary. Do not spray 70% Ethanol in a BSC when the blower is running as the Lower Explosive Limit (LEL) of Ethanol is quickly reached; vapor may be drawn through the motor (an ignition source) and cause a fire. Instead, wet a paper towel outside of the BSC and wipe down surfaces. Ten percent bleach is highly corrosive to stainless steel and should not be used.
4. Wipe down all materials with 70% Ethanol before placing in the BSC. This simple step will help minimize the introduction of mold spores and other contaminants and minimize contamination of your cultures. Further reduction of the microbial load on materials in BSCs may be achieved by periodic decontamination of incubators and refrigerators.

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5. Line the work surface of the BSC with absorbent matting to contain any spills that might occur. Make sure it does not obstruct the grills and tape the corners to prevent movement while in use.
6. Place decontaminated materials in the BSC before beginning work to minimize disruptions to the fragile air curtain. Movement of hands or arms in a sweeping motion into and out of the cabinet will disturb the air curtain and create a non-sterile working environment. Move arms in and out slowly, perpendicular to the face opening of the cabinet, to reduce this risk. Other personnel activities in the room (e.g. foot traffic behind a BSC operator, room fans, opening/closing room doors) may also disrupt the air curtain and are to be avoided.
7. Segregate clean items from those that will become contaminated. Work from “clean” to “dirty” in the BSC. Place materials toward the back, but do not block the rear grille.
8. If there is a drain valve under the BSC, make sure it is closed prior to beginning work.
9. If the BSC is equipped with an alarm, test the alarm and switch it to the “ON” position. Never operate a BSC while a warning light or alarm is on.
10. Operate the BSC blower fan for five minutes to allow the cabinet to purge and clear particulates suspended in the cabinet.
11. Lift the sash to recommended height.
12. Hold a Kimwipe to confirm inward air flow in the middle of the BSC.
13. Adjust the stool height so the operator’s face is above the front opening.
14. Lab coats are worn buttoned over street clothing; vinyl, nitrile or other suitable gloves are worn for hand protection; eye protection is worn to protect the operator against splashes to the eyes.
15. Delay manipulation of materials for at least one minute after placing hands/arms inside the cabinet. This will allow the cabinet to stabilize, to “air sweep” the hands and arms, and allow time for reduction of turbulence inside the BSC. Perform all work using a limited number of slow movements, since quick movements will disrupt the air barrier.
16. Take care not to block the front grille with absorbent matting, research notes, discarded pipette wrappers, pipetting devices, etc.
17. When the user’s arms rest flat across the front grille, they block the grille opening and contaminated room air may flow directly into the work area. Raising the arms slightly off the grille will correct this problem.
18. Perform all tasks in the BSC at least four inches from the inside edge of the front grille for proper protection of your work. Protection is optimal toward the middle of the BSC.

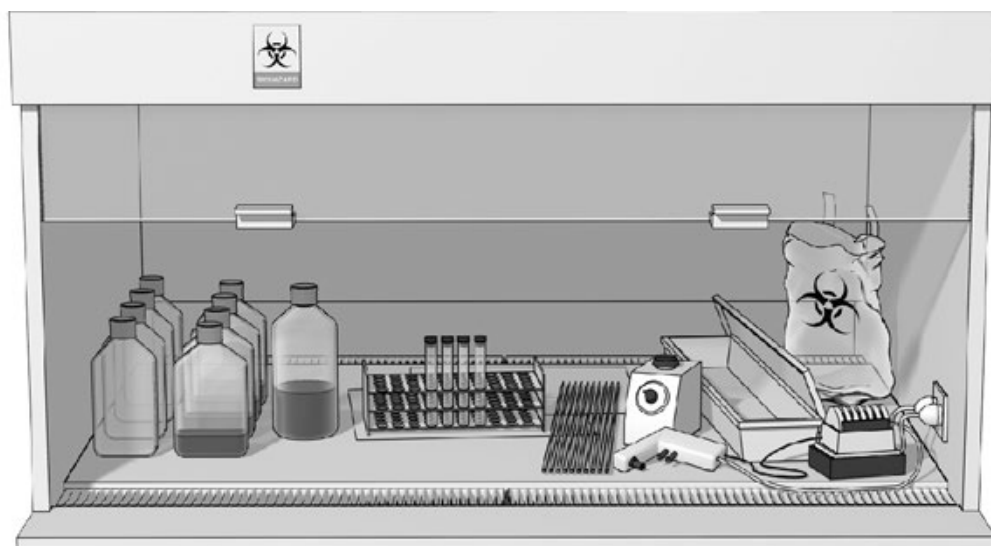
Material Placement in the BSC

1. Materials and equipment placed inside the BSC cause disruption in the airflow. The higher the profile of each piece, the greater the disruption. The objective is to keep this disruption to a minimum. Use the fewest possible supplies in the BSC and maintain the lowest profile possible for each. This will help reduce turbulence and possible cross-contamination, or an outright breach of containment.
2. Store extra supplies (e.g., additional gloves, culture plates or flasks, culture media) on a lab

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cart outside the cabinet. Only the materials and equipment required for the immediate work should be placed in the BSC.

3. Plastic-backed absorbent matting can be placed on the flat work surface but not over the front or rear grille openings. Anchor the matting well to prevent movement and possible blocking of the grilles. The use of matting will facilitate routine cleanup. When grossly contaminated or at the end of the day, it can be folded and disposed in a biohazard bag or biohazard waste disposal box. Minor spills can be spot-decontaminated with 70% Ethanol.
4. The workflow should be from “clean to dirty”. To prevent contamination, place materials and supplies in the cabinet in a way that limits the movement of “dirty” items over “clean” ones.
5. Materials should be placed at the side or as far back in the cabinet as practical, toward the rear of the work surface but not blocking the rear grille.



6. Aerosol-generating equipment (e.g., vortex mixers, benchtop centrifuges) should also be placed toward the rear of the cabinet. Keep bulky items such as biohazard bags and discard pipette trays to the “dirty” side in the cabinet.
7. The correct sash position (usually 8” or 10” above the base of the opening) should be indicated on the front of the cabinet. If the sash must be lifted to accommodate equipment such as a bench-top centrifuge, return it to the proper height before beginning work. On most BSCs, an audible alarm will sound if the sash is in the wrong position while the fan is operating.
8. Certain common practices interfere with proper operation of the BSC. For example, movement in and out of the BSC creates turbulence which disrupts the integrity of the air barrier, compromising protection of both personnel and product. To minimize turbulence, it is important to observe the following: do not tape the biohazard waste bag to the outside of the cabinet or use upright pipette collection containers either in or outside the BSC. Use only horizontal pipette discard trays containing an appropriate chemical disinfectant inside

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- the BSC. Place the trays on the “dirty” side of the cabinet.
9. Contaminated materials should not be brought out of the cabinet until they have been surface-decontaminated.
 10. The BSC should be left empty at the end of the day. It is to be used for work, not storage.

VIDEO:

Working Safely BSC: Working With and In Your NuAire Biological Safety Cabinet

<https://www.youtube.com/watch?v=Hos8O55cA>