Purpose

- To provide details for the safe operation of biological safety cabinets (BSCs) and ensure adequate containment of biohazards.
- Biological safety cabinets shall be used:
    a. For handling biohazardous materials at BSL-2 to protect lab workers from accidental exposures.
    b. To provide the clean environment necessary for propagation of cell cultures.
- All users of BSCs shall follow the procedures described below.

Basic Safety Guidelines

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, 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 wall 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.

See Appendix A, Biosafety Cabinets, in the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories, 5th Ed.* https://www.cdc.gov/labs/BMBL.html for airflow patterns of the four BSC types (Type A1, Type A2, Type B1 or Type B 2). If the type is not identified on the front of your BSC, you can find it on the metal plate that includes the model and serial number.

Become familiar with the airflow pattern for your particular type of 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: https://learn.nuaire.com/lab-equipment-videos

STANDARD OPERATING PROCEDURES

Preparing for Work within a Class II BSC

1. Have a written checklist of materials needed for a particular activity.
2. 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 such as Wescodyne, an iodophor, or a quaternary ammonium compound. Several applications of 70% Ethanol may be necessary. Do not spray 70% Ethanol in a BSC when the blower is running as the LEL of Ethanol is quickly reached; vapor may be drawn through the motor (an ignition source) and cause a fire. Ten percent bleach is highly corrosive to stainless steel and should only be used with great care and rinsed multiple times with sterile water. Wiping with non-sterile water may re-contaminate the cabinet.
3. Wipe down all materials with 70% Ethanol before placing in the BSC. This simple step will help reduce introduction of mold spores and other contaminants and minimize contamination of cultures. Further reduction of the microbial load on materials in BSCs may be achieved by periodic decontamination of incubators and refrigerators.
4. Place decontaminated materials in the BSC before beginning work to minimize disruptions to the fragile air curtain inside the BSC. 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. walking traffic behind a BSC operator, room fans, opening/closing room doors) may also disrupt the air curtain and should be avoided.
5. Segregate clean items from those that will become contaminated. Work from “clean” to “dirty” in the BSC. Place materials toward the back of the BSC, but do not to block the rear grille.
6. If there is a drain valve under the BSC, make sure it is closed prior to beginning work.
7. 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.
8. Operate the BSC blower fan for five minutes to allow the cabinet to purge and clear particulates suspended in the cabinet.
9. Lift the sash to recommended height.
10. Tape or hold a Kimwipe to confirm inward air flow at the middle of the BSC.
11. Adjust the stool height so the operator’s face is above the front opening.
12. Lab coats are worn buttoned over street clothing; latex, vinyl, nitrile or other suitable gloves are worn for hand protection; eye protection is worn to protect the operator against eye splashes.
13. 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.
14. Take care not to block the front grille with absorbent matting, research notes, discarded wrappers, pipetting devices, etc.

15. 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.
16. 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 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 contaminated or at the end of the day, it can be folded and disposed in a biohazard bag or biohazard waste disposal box.
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, tabletop 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 has to be lifted to accommodate equipment, 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 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.

Hazards

1. Many procedures conducted in BSCs can create aerosols or splatter. Use good microbiological techniques when working to minimize generation of splatter and aerosols and reduce the risk of exposure to infectious materials.
2. Keep clean materials at least one foot away from aerosol-generating activities in the BSC to minimize the potential for cross-contamination.
3. To reduce contamination, do not hold opened tubes or bottles in a vertical position. Hold lids of Petri dishes and tissue culture plates above them to shield the contents. Do not place bottle or tube caps on the matting. Recap or cover items as soon as possible.
4. Open flames are prohibited in biological safety cabinets at URI. On an open bench, flaming the neck of a culture vessel will create an upward air current that prevents microorganisms from falling into the tube or flask. However, an open flame in a BSC creates turbulence inside the BSC that disrupts the pattern of HEPA-filtered air supplied to the work surface, compromising the air curtain, creating an unsafe condition. Open flames can also damage HEPA filters in the BSC. When absolutely necessary, touch-plate micro burners equipped with a pilot light to provide a flame on demand may be used. Cabinet air disturbance and heat buildup will be minimized. Turn the burner off as soon as work has been completed. Small electric "furnaces" or micro incinerators are available for decontaminating bacteriological loops and needles, and are preferred inside the BSC. Use disposable or recyclable sterile loops when possible.
5. To protect vacuum pumps and personnel who service them, use a suction flask connected to an overflow flask. Insert an in-line HEPA or equivalent filter just before the line connects to the vacuum pump. Flasks should be in secondary containment to prevent breakage.
6. Aspirated materials are inactivated by adding a chemical decontaminant to the flask. Once inactivation is complete, liquid materials can be disposed as noninfectious waste by flushing down the sink with copious amounts of water. If using 10% bleach, change the flask at least once a week, but preferably twice a week as the bleach breaks down quickly and loses its effectiveness in a short period of time.
7. The PI must determine the appropriate method of decontaminating materials removed from the BSC when work has been completed. This information must be described in the lab's "Safe Use of the Biosafety Cabinet SOP". Contaminated items can be placed in a biohazard bag prior to removal from the BSC.
8. Decontaminate the exterior surfaces of items, including biohazard waste bags, just prior to removing from the cabinet and dispose waste in the biohazard waste disposal box. Wipe down (don't spray) with 70% Ethanol.
9. Used pipettes are decontaminated in the tray inside the BSC. Do not dispose them in the biohazard waste box. Instead, dispose them in a cardboard box lined with a plastic bag to contain any residual disinfectant from the tray. When the box is full, tape it up and carry it out to the Dumpster for disposal in the solid waste stream. Change the disinfectant in the tray frequently.

Operation

1. BSCs are designed to be used by a one person at a time. Multiple users will disturb the air curtain within the cabinet, greatly reducing its containment capabilities and creating an unsafe work environment.
2. If the BSC is located next to a door, the door should be kept closed when the BSC is in use. Also, the BSC should not be positioned directly under an air duct as this will also disturb the air curtain.
3. All operations should be performed on the work surface, at least four inches in from the front grille but preferably toward the middle of the BSC for optimal protection.

Decontamination

1. Clean up small spills in the BSC when they occur. Remove contaminated absorbent matting and dispose it in a biohazard waste box. Clean up splatter on the sides of the cabinet with a towel dampened with an appropriate decontaminating solution. Change gloves before placing clean absorbent matting in the cabinet. Wash hands whenever gloves are changed or removed.
2. Spills large enough to cause liquids to flow through the front or rear grilles require more extensive decontamination. See the University's Spill Management Plan for specifics.
3. If the spilled liquid contains radioactive material, a similar procedure can be followed. Contact the Radiation Safety office for specific instructions and have a spill response plan in place before an incident occurs.
4. When you have finished working and with the BSC blower still running, decontaminate all containers and equipment. Wipe down the work surface, the sides and back of the BSC, and the inside of the glass with 70% Ethanol. Do not spray Ethanol inside a BSC.
5. Turn the BSC blower off.
6. Remove gloves and lab coat in a way that prevents contamination of unprotected skin. Wash hands as the final step in safe microbiological practices.

Ultraviolet Light Use

Statement from National Institutes of Health (NIH)

Use of Ultraviolet (UV) Radiation in Laboratories

"The NIH does not recommend or support the use of ultraviolet (UV) radiation in laboratories. Although UV is effective against most microbes, it requires an understanding of its abilities and limitations. The 253.7-nm wavelength emitted by the germicidal lamp has limited penetrating power and is primarily effective against unprotected microbes on exposed surfaces or in the air. It does not penetrate soil or dust. The intensity or destructive power decreases by the square of the distance from the lamp. Thus, exposure time is always related to the distance. The intensity of the lamp diminishes over time. This requires periodic monitoring with a UV

meter. The intensity of the lamp is drastically affected by the accumulation of dust and dirt on it. The bulbs require frequent maintenance. In addition, there are safety hazards associated with the use of UV that require personal protective equipment or other safety devices to protect users. UV lights in biosafety cabinets require the cabinet be decontaminated prior to performing maintenance on the system. Past experience has proven that good techniques in conducting experiments are highly effective in preventing contamination. The use of UV radiation does not eliminate the necessity for using good practices and procedures."

http://www.ors.od.nih.gov/sr/dohs/BioSafety/decon/Pages/decontamination.aspx

Other Safety Considerations:

1. If a BSC is malfunctioning, do not attempt to use it. Post a sign to indicate the cabinet is out of service and notify the PI. An outside vendor will need to be brought in to repair and re-certify the unit.

   Call EHS at (401) 874-7019 to schedule a service call from an NSF-certified biosafety cabinet testing firm.

   The PI is responsible for all costs unless the equipment is still under warranty.
2. Close BSC sash when not in use.
3. Keep BSCs clean and clean up minor spills as they occur. Decontaminate the BSC properly after each use.
4. If the BSC alarm begins to sound while you are using it, or if there is a power failure, secure all biohazardous materials, close the sash and leave the room. If the room lights are still on, the PI needs to initiate a service call. If there has been a general power failure, raise the sash as soon as power has been restored, and allow the cabinet to purge for 5 minutes before using again.

Inspection and Certification:

1. Biological Safety Cabinets are certified annually and certification tags posted on the equipment.
2. PI's are responsible for annual certification.
3. Do not use a BSC unless the certification is up to date.