Helpful Hints on the Use of Biological Safety Cabinets

A Biological Safety Cabinet (BSC) is a valuable supplement to good sterile technique and a necessary containment device when working with biohazardous agents. If the BSC is not operated correctly, it will not provide adequate protection. The Department of Health & Human Services, Centers for Disease Control and Prevention, and National Institutes of Health have authored a document on proper use of BSCs. The document (Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets) is a valuable resource when working in a BSC. Much of the information in this Helpful Hints document comes from the more thorough CDC document available at: [http://www.cdc.gov/OD/OHS/biosfty/primary_containment_for_biohazards.pdf](http://www.cdc.gov/OD/OHS/biosfty/primary_containment_for_biohazards.pdf).

Detailed information on OSEH services for BSCs can be found in OSEH’s Biological Safety Cabinet guideline ([http://www.oseh.umich.edu/pdf/guideline/guidbsc.pdf](http://www.oseh.umich.edu/pdf/guideline/guidbsc.pdf)) and the OSEH Engineering Controls Standard of Care document set ([http://www.oseh.umich.edu/guideline.html](http://www.oseh.umich.edu/guideline.html)).

---

**Start-up and Set-up**

1. Although BSCs are designed for 24 hours per day operation, *this is not required nor recommended.* Some investigators find that continuous operation helps to control the laboratory’s level of dust and other airborne particulates at the expense of energy conservation. If the cabinet has been shut down, the blowers should be operated at least four minutes before beginning work to allow the cabinet to “purge.” This purge will remove any suspended particulates in the cabinet.

2. Check that the vertical sliding window sash is at the correct height. The operating position is not variable on BSCs. If the sash is positioned too high, the user is more likely to contaminate themselves and their work. If the sash is positioned too low, the unit is starved for airflow creating the likelihood of product contamination.

3. Make sure you have airflow, either by listening for blower sound or by feeling the airflow with your fingers. Check that your BSC’s gauges and indicators have appropriate readings.

4. If your cabinet is equipped with a U.V. light, turn it off and turn on the fluorescent light before beginning work. It is important to avoid hazardous exposure of skin and eyes to U.V. light.

5. Make sure the drain valve is closed.

6. Wipe down all the interior surfaces of the BSC with an appropriate disinfectant. The work surface, the interior walls (except the supply filter diffuser), and the interior surface of the window should be wiped with 70% ethanol (EtOH), a 1:10 dilution of household bleach (i.e., 0.05% sodium hypochlorite), or other disinfectant as determined by the investigator to meet the requirements of the particular activity. When bleach is used, a second wiping with sterile water is needed to remove the residual chlorine, which may eventually corrode stainless steel surfaces.

7. Place all the equipment and supplies you will need inside the cabinet to minimize entering and exiting. The surfaces of all materials and containers placed into the cabinet should be wiped with 70% EtOH to reduce the introduction of contaminants to the cabinet environment. This includes placing a receptacle for waste and used pipettes inside the BSC. Segregate items that will remain clean from the ones that will get contaminated. All materials should be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the
cabinet. Similarly, aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) should be placed toward the rear of the cabinet. Bulky items such as biohazard bags, discard pipette trays and vacuum collection flasks should be placed to one side of the interior of the cabinet.

8. Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet.

---

**Working in the Cabinet**

1. BSCs are designed for a single operator. Never work two or more people at a time in any BSC. Multiple users will cause air disturbance and destroy the containment capabilities of the BSC.
2. Never operate a cabinet while a warning light or alarm is on. Call OSEH at 3-6973 to service failing BSCs.
3. The operator should be seated with armpits level with the bottom of the sash.
4. Make sure everything necessary for the procedure is already inside and sterile. Slowly introduce gloved hands into the BSC and let air wash over them for a few seconds before beginning. Perform all work using a limited number of slow movements, as quick movements disrupt the air barrier.
5. Keep all materials at least four inches inside the sash opening. Never place items on the front or rear perforated grills. This creates air turbulence that increases the risk of contamination to the user or the research.
6. Activities that create eddy currents (opening and closing doors and windows, personnel walking near the cabinet), should be minimized as these types of activities can disrupt the air barrier.
7. Avoid movements in and out of the cabinet during the procedure.
8. The work flow should be from “clean to dirty”. Materials and supplies should be placed in the cabinet in such a way as to limit the movement of “dirty” items over “clean” ones.
9. Opened tubes or bottles should not be held in a vertical position. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impaction of downward air. Bottle or tube caps should not be placed on the toweling. Items should be recapped or covered as soon as possible.
10. Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current which prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence which disrupts the pattern of HEPA-filtered air being supplied to the work surface. When deemed absolutely necessary, touch-plate microburners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric “furnaces” are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable or recyclable sterile loops should be used whenever possible.
11. Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter. This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to inactivate the
microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste. See image below.

![Diagram of vacuum system](image)

One method to protect a house vacuum system during aspiration of infectious fluids. The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask serves as a fluid overflow collection vessel. An in-line HEPA filter (C) - Whatman Vacu-Guard® is used to protect the vacuum system (D) from aerosolized microorganisms and overflow.

---

**Completing Work in the Cabinet**

1. Investigators must determine the appropriate method of decontaminating materials that will be removed from the BSC at the conclusion of the work. When chemical means are appropriate, suitable liquid disinfectant should be placed into the discard pan before work begins. Items should be introduced into the pan with minimum splatter and allowed appropriate contact time as per manufacturer’s instructions. Alternatively, liquids can be autoclaved prior to disposal. Contaminated items should be placed into a biohazard bag, discard tray, or other suitable container prior to removal from the BSC.

2. All equipment that has come in contact with the research agent should be decontaminated. The cabinet should be allowed to run for at least three minutes with no activity so that the airborne contaminants will be purged from the work area. Then remove the equipment.

3. After all items have been removed, wipe the interior surfaces with a disinfectant.
Common Errors to Avoid

1. Keep papers, paper towels, Kim Wipes, work surface diapers, vials, or any other objects from being pulled in the back, front, or side slots or grills. These items can damage your cabinet’s internal components.
2. Do not store equipment or supplies in the cabinet.
3. Do not use the top of the cabinet for storage. The HEPA filter could be damaged and the airflow disrupted.
4. Never disengage the alarm. It indicates improper airflow and reduced performance that may endanger the researcher or the experiment.

BSC Placement, Utilities, Maintenance, UV, and Decontamination

BSC Placement
BSCs provide personnel, environmental and product protection during the manipulation of infectious microorganisms. Certain considerations must be met to ensure maximum effectiveness of these primary barriers. Adequate clearance must be provided behind and on each side of the BSC to allow easy access for maintenance and to ensure that BSC operation is not hindered. A 12 to 14 inch clearance above the cabinet may be required to provide for accurate air velocity measurement across the exhaust filter surface and for exhaust filter changes.

The ideal location for the biological safety cabinet is remote from the entry (i.e., the rear of the laboratory away from traffic), since people walking parallel to the face of a BSC can disrupt the air curtain. Open windows, air supply registers, portable fans or laboratory equipment that creates air movement should not be located near the BSC. Similarly, chemical fume hoods must not be located close to BSCs. Call OSEH at 3-6973 for assistance in BSC placement.

Utility Services
UM Maintenance personnel will not provide natural gas service to BSCs without written approval from the UM Biological Safety Officer. Contact OSEH at 3-6973.

Maintenance
Most BSC maintenance, warranty repairs, and certifications are performed by OSEH technicians. OSEH has written Standard of Care documents that describe these provided services. The documents can be reviewed at: http://www.oseh.umich.edu/guideline.html. See the Engineering Control Standard of Care document set.

Ultraviolet Lamps
Ultraviolet (UV) lamps are not required in BSCs nor are they necessary. If installed, UV lamps must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the ultraviolet light. The lamps should be checked weekly with a UV meter to ensure that the appropriate intensity of UV light is being emitted. UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer. If the cabinet has a sliding
sash, close the sash when operating the UV lamp. The laboratory is responsible to monitor, purchase, and replace UV bulbs.

**Decontamination**

BSCs that have been used for work involving infectious materials may require decontamination before HEPA filters are changed, the BSC is moved, or internal repair work is done. A risk assessment considering the agents manipulated within the BSC must be performed to determine the need and method for decontamination. In order to conduct this risk assessment the investigator must complete the OSEH Biological Safety Cabinet Clearance form and submit it to the UM Biological Safety Officer for review. [http://www.oseh.umich.edu/pdf/deconform.pdf](http://www.oseh.umich.edu/pdf/deconform.pdf).

---

**Air Flow Patterns in Class II Biological Safety Cabinets**

Please see the diagrams below depicting the airflow patterns in the various Class II BSCs.

- Class II, Type A1 BSC
- Class II, Type B1 BSC
- Class II, Type A2 BSC
- Class II, Type B2 BSC

---

The Class II, Type B1 BSC (classic design). A. front opening, B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. negative pressure dedicated exhaust plenum, F. blower, G. additional HEPA filter for supply air. Note: The cabinet exhaust needs to be hard connected to the building exhaust system.
The tabletop model of a Class II, Type A2 BSC. A. front opening, B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. positive pressure common plenum, F. negative pressure plenum. The Class II Type A2 BSC is not equivalent to what was formerly called a Class II Type B3 unless it is connected to the building exhaust system. Note: The Type A2 BSC can be connected to the building exhaust with the use of a manufacturer supplied canopy connection.

The Class II, Type B2 BSC. A. front opening, B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. negative pressure exhaust plenum, F. filter screen. Note: The carbon filter in the exhaust system is not shown. The cabinet needs to be hard connected to the building exhaust system.