

 <p>UBC Biosafety Committee 102 - 6190 Agronomy Rd. Vancouver BC, V6T 1Z3</p>	Standard Operating Procedure	IBC-SOP-002
	General Use	<p>Effective date: November 20, 2013. Review date: April 30, 2014. Supersedes: N/A.</p>

Safe Use of a Class II Biological Safety Cabinet

1. SCOPE

This document will describe how to work in a Class II Biological Safety Cabinet (BSC)

2. RESPONSIBILITY

Prior to working in a BSC, every worker must be appropriately trained. It is the responsibility of the supervisor to ensure that workers are aware of:

- How a BSC works
- What a BSC is used for
- When and how often a BSC needs to be certified
- What the safe practices are when working with a BSC

After the training takes place, the training must be documented in accordance to WorkSafeBC, CFIA and PHAC regulations.

3. PERSONAL PROTECTIVE EQUIPMENT

When working in a BSC, the operator should wear

- closed-front over garment (e.g. surgical gown with full-length sleeves or fully buttoned lab coat)
- gloves (latex or vinyl gloves) Note: Gloves should overlap the cuffs to ensure that aerosols do not contaminate the hands, arms and surfaces.
- long pants or skirt and full covering shoes

4. MATERIALS

Prior to working in a BSC, ensure that you:

- prepare a written checklist of materials necessary for your particular protocol (this would only be required for the first time the protocol was followed)
- have protocols written out and accessible (this may be in a lab book, protocol book, or online).
- have determined the materials that need to be placed in the BSC and which

materials should be placed outside. This will minimize the in-and-out motions that could affect the protective barrier of the BSC.

- Ensure that the BSC you are working with is appropriate for your protocols. For instance, if you are working with radioisotopes or volatile chemicals, ensure that you have selected the correct BSC type.

5. REFERENCES AND DEFINITIONS

[UBC Laboratory Biosafety Manual](#)

6. PROCEDURE

6.1. BSC Start-Up Procedure

- 6.1.1. If a UV light is being employed, turn it off first
- 6.1.2. Turn on the BSC and open the sash to the appropriate sash height
- 6.1.3. Cabinet blowers should be operated at least ten to fifteen minutes before beginning work to allow the cabinet to "purge". This purge will remove any particulates in the cabinet.
- 6.1.4. Ensure that nothing is blocking the front grilles
- 6.1.5. The work surface, the interior walls (not including the supply filter diffuser), and the interior surface of the window should be wiped with either
 - 70% ethanol (EtOH) or,
 - other disinfectant as determined by the investigator to meet the requirements of the particular activity

Note: When bleach is used, a second wiping with sterile water is needed to remove the residual chlorine, which may eventually corrode stainless steel surfaces. Wiping with non-sterile water may re-contaminate cabinet surfaces, a critical issue when sterility is essential (e.g., maintenance of cell cultures).

6.2. While working in a BSC

- 6.2.1. After the BSC has been sufficiently purged and decontaminated, the following practices should be employed to maintain product, personnel and environment protection.
- 6.2.2. **Arm Movements:** While working in a BSC, it is imperative that errant air flow velocities are not introduced for the proper functioning of the BSC.
 - 6.2.2.1. Once hands/arms are placed inside the cabinet, manipulation of materials should be delayed for approximately one minute. This allows the cabinet to stabilize and to "air sweep" the hands and arms to remove surface microbial contaminants.
 - 6.2.2.2. Move arms in and out slowly, perpendicular to the face opening of the cabinet

- 6.2.2.3. Ensure that rapid arm movements in sweeping motions are minimized. This movement will disrupt the air curtain and may compromise the partial barrier containment that is provided by the BSC.
- 6.2.3. **Front Grille:** To ensure that the BSC can provide proper product, personnel and environment protection, it is important that the front grilles are not blocked.
 - 6.2.3.1. Raise arms slightly to ensure that arms are not resting on the grille.
 - 6.2.3.2. Ensure other items are not blocking the grille (ie protocols, pipettes etc.)
- 6.2.4. **Placement of materials inside the BSC:** Materials or equipment placed inside the cabinet may cause disruption to the airflow, resulting in turbulence, possible cross-contamination, and/or breach of containment.
 - 6.2.4.1. 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 simple step will reduce introduction of mold spores and thereby minimize contamination of cultures.
 - 6.2.4.2. Only the materials and equipment required for the immediate work should be placed in the BSC
 - 6.2.4.3. Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet.
 - 6.2.4.4. All operations should be performed at least four "4" inches from the front grille on the work surface
 - 6.2.4.5. Active work should flow from the clean to contaminated area across the work surface.
- 6.2.5. **Microbiological Techniques:** Many common procedures conducted in BSCs may create splatter or aerosols. Good microbiological techniques should always be used when working in a biological safety cabinet. For example, techniques to reduce splatter and aerosol generation will minimize the potential for personnel exposure to infectious materials manipulated within the cabinet. Class II cabinets are designed so that horizontally aerosol spores will be captured by the downward flowing cabinet air within fourteen inches of travel.
 - 6.2.5.1. Keep clean materials at least one foot away from aerosol-generating activities. This will minimize the potential for cross-contamination.
 - 6.2.5.2. The general work flow should be from "clean" to contaminated "dirty". Materials and supplies should be placed in such a way as to limit the movement of "dirty" items over "clean" ones.

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- 6.2.5.3. 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.
- 6.2.5.4. Bottle or tube caps should not be placed on the toweling.
- 6.2.5.5. Items should be recapped or covered as soon as possible.

6.2.6. **Biohazard bags and other waste containers:** The frequent inward/outward movement needed to place objects in biohazardous bags and pipette collection trays is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. The following describes specific practices to use when working with either of these items:

6.2.6.1. **Biohazard bags:** Typically used when contaminated waste is going to be autoclaved.

- 6.2.6.1.1. Ensure that the correct type of bag is used for the correct biohazard
- 6.2.6.1.2. To minimize the chance of leaks, double bag
- 6.2.6.1.3. The bag should be placed to one side of the interior of the cabinet and not taped to the outside of the cabinet.
- 6.2.6.1.4. Water should be placed within the bag to allow steam to be generated during the autoclave cycle
- 6.2.6.1.5. Materials that are contaminated must be placed into the bag and the bag must be **sealed** prior to it being removed from the cabinet.
- 6.2.6.1.6. The bag should be transported and autoclaved in a leak proof tray or pan.

6.2.6.2. **Discard trays or pans:** Only horizontal pipette discard trays or pans should be used within the cabinet. Upright pipette collection containers should not be used in BSC's nor placed on the floor outside the cabinet.

- 6.2.6.2.1. Practices to use when discard trays and pans are decontaminated using chemical disinfectants:
 - Discard pipette trays should be placed to one side of the interior of the cabinet.
 - Items should be introduced into the pan with minimum splatter, and allowed appropriate contact time as per manufacturer's instructions.
 - The discard pan should be covered and surface decontaminated in the BSC prior to removal out of the cabinet.

6.2.6.2.2. Practices to use when discard trays and pans are decontaminated using the autoclave:

- Discard pipette trays should be placed to one side of the interior of the cabinet.
- Water should be added to the bag or tray prior to autoclaving, to allow for steam to be generated through the autoclave cycle.
- Items should be introduced into the pan with minimum splatter.
- The tray needs to be sealed prior to removal from the cabinet.

6.2.7. **Absorbent Toweling:** Plastic-backed absorbent toweling can be placed on the work surface (but not on the front or rear openings). This toweling facilitates routine cleanup and reduces splatter and aerosol formation during an overt spill. It can then be folded and placed in an autoclavable biohazard bag when work is completed.

6.2.8. **Aerosol generating equipment:** Aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) should be placed toward the rear of the cabinet to take advantage of the air split that occurs in the BSC. The downward moving air "splits" as it approaches the work surface; the blower draws part of the air to the front grille and the remainder to the rear grille.

6.2.9. **Open Flames:** 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 that prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence that disrupts the pattern of air 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 sterile loops can also be used.

6.2.10. **Aspirator bottles or suction flasks (UBCV-RMS-OHS-GDL-14-002):** 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. The flasks and aspirator bottles, if kept in the BSC, must be kept to one side of the cabinet. 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 kill the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of appropriately as noninfectious waste.

6.2.11. Biohazardous Spills: Small contained spills on the work surface can be handled as outlined in the SOP: IBC-SOP-0001, Biological Spill Clean-up. Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination.

- 6.2.11.1. All items within the cabinet should be surface decontaminated and removed.
- 6.2.11.2. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan.
- 6.2.11.3. Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. Manufacturer's directions should be followed.
- 6.2.11.4. The spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag.
- 6.2.11.5. The drain pan should be emptied into a collection vessel containing disinfectant. A flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. This procedure serves to minimize aerosol generation.
- 6.2.11.6. The drain pan should be flushed with water and the drain tube removed.
- 6.2.11.7. Gloves should be disposed of and hands must be washed.

Note: Should the spilled liquid contain radioactive material, a similar procedure can be followed. Radiation safety personnel should be contacted for specific instructions.

6.2.12. Power Failure while working in the BSC: When a power failure occurs while you are working in the BSC, the following procedures must be employed.

- 6.2.12.1. Seal all open containers
- 6.2.12.2. Dispose of gloves within the BSC
- 6.2.12.3. If the BSC has a movable sash, bring it down to the closed position.

6.3. BSC shut down procedures:

6.3.1. After work is completed in the cabinet, the following procedures should be followed:

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- 6.3.1.1. Allow the cabinet to run for 5 minutes with no activity
- 6.3.1.2. All containers and equipment should be surface decontaminated prior to removal
- 6.3.1.3. Remove gloves and dispose of them as appropriate. Wash your hands.
- 6.3.1.4. Put on clean gloves and ensure that all contaminated materials have been appropriately disposed of in the biohazardous bag or discard tray. Seal and surface decontaminate biohazardous bags and waste containers prior to their removal.
- 6.3.1.5. Decontaminate the work surface using an appropriate disinfectant (ie. 70% ethanol)
- 6.3.1.6. At the end of the workday, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back, and the interior of the glass.
- 6.3.1.7. Remove gloves and gowns and wash hands.

7. REVIEW AND RETENTION

This SOP is reviewed annually or whenever deemed necessary by the responsible departmental representative, the Biosafety Office in Risk Management Services.

Amendments:

Any amendments to this document must be noted in Section 3.2 of your Biosafety Permit Application. Otherwise this SOP may be documented by its SOP#.

8. DOCUMENT APPROVAL SIGNATURES

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Revised By:			
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