

Biological Safety Cabinet Standard Operating Procedure

1. Definition:

- a. In general, all laboratory activities involving human tissues, isolated cells, blood, and blood fractions must be conducted at a minimum of level 2 containment conditions (CL2). Biological safety cabinets (BSCs) are required for level 2 or greater containment laboratories. Class II BSCs provide the microbe-free work environment necessary for cell culture propagation and handling of infectious organisms.
- b. BSCs protect the user, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating substances containing infectious agents, such as viruses, bacteria and primary tissue cultures.
- c. BSCs use HEPA filters, a filter that traps 99.97% of particles of 0.3 μm in diameter and 99.99% of particles of greater or smaller size, thus capturing all infectious agents and ensuring only microbe-free air is exhausted from the cabinet or directed to the work surface.

2. Responsibility:

- a. Managers, or Supervisors or Principal Investigators shall:
 - Ensure that only workers who are informed about hazards, controls, safe work and emergency procedures can use the BSC.
 - Provide and maintain appropriate equipment and materials to work safely.
 - Ensure that all appropriate precautions are being followed and that required personal protective equipment (PPE) is being worn.
- b. Workers shall:
 - Work in accordance with the standard and emergency operating procedures.
 - Ensure the BSC is in good condition before use – immediately report any issues.
 - Wear appropriate PPE as required.

3. Prior to Operation:

- a. Read the Safety Data Sheets (SDS) for materials being used in a BSC. Note any precautions regarding the use of the chemical or microorganisms in the BSC.
- b. Lift the sash to the recommended height.
- c. Turn on the BSC fan 10 minutes before beginning work to allow adequate air filtration.
- d. Check the air intake and exhaust grilles for obstructions, and check the pressure gauge reading indicating HEPA filter load. If you do not know the acceptable reading for the cabinet, ask the certifier or consult your manufacturer's manual.
- e. If the cabinet is equipped with an alarm, test the alarm and switch it to the "ON" position.
- f. Confirm inward airflow by holding a piece of tissue at the middle of the edge of the viewing panel and ensuring it is drawn in.
- g. Decontaminate the cabinet surface and surface-decontaminate all materials to be placed inside the BSC (refer to the Cleaning and Disinfection section).

- h. Bulky items, such as waste containers and suction collection flasks, should be placed to one side of the interior of the cabinet.
- i. Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may impede proper airflow and the level of protection provided. Consider using wire racks or shelving to increase airflow around front and rear grills.
- j. Wear eye protection, a lab coat, closed-toe shoes and disposable gloves to protect yourself as well as your samples from contamination.
- k. Respirators may be required for some procedures. The nature of the Personal Protective Equipment (PPE) worn may vary according to the findings obtained from the biological risk assessment.

NOTE: Avoid making quick movements past the BSC, or pulling your hands quickly in or out of the BSC as this disrupts airflow and can cause puff-back.

4. BSC Operation:

- a. Active work should flow from clean to contaminated areas across the work surface. See Figure 1.

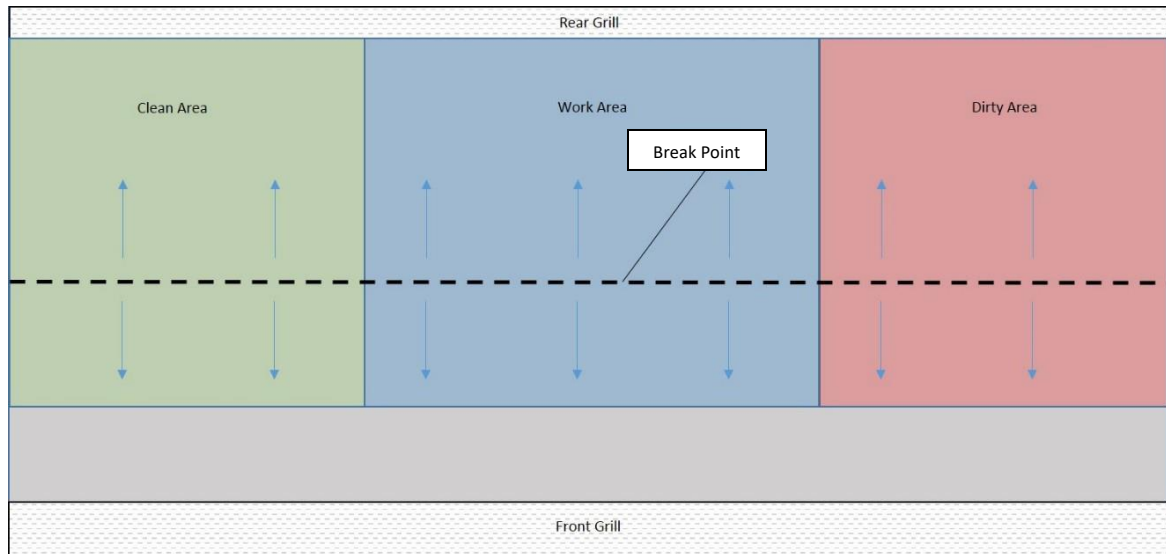


Figure 1: BSC workspace setup. Work should be performed at or behind the break point, from the clean area to the dirty area.

- b. Arms should be moved in and out slowly, perpendicular to the front opening to minimize disruption of the air curtain and laminar flow. Quick movements can cause puff-back.
- c. Work as far to the back as possible, but within comfortable reach.
- d. Always use mechanical pipetting aids. Mouth pipetting is prohibited.
- e. Heat sources such as Bunsen burners are strictly prohibited inside the BSCs as they significantly disrupt the laminar flow of air.
- f. To sterilize bacteriological loops, micro-burners or electric “furnaces” may be used.

- g. Locate liquid waste traps inside cabinet and use an in-line HEPA filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container (such as a durable plastic tray or box) to prevent breakage.

5. Cleaning and Disinfection:

- a. When work is completed, all equipment and supplies from the BSC should be decontaminated and removed from the cabinet.
- b. The interior surfaces should also be wiped with an appropriate disinfectant that would kill any microorganisms that could be found in the cabinet. Corrosive chemicals such as bleach should be avoided, but if used, should be followed with a wipe down of sterile water or 70% ethanol.
- c. Allow the cabinet to run for 10 minutes.

6. Spills:

- a. Alert the other laboratory employees.
- b. Leave the cabinet turned on.
- c. While wearing gloves, spray or wipe cabinet walls, work surfaces and equipment with disinfectant equivalent to 1:10 bleach solution. If necessary, flood the work surface, as well as drain-pans and catch basins below the work surface, with disinfectant for a contact time of at least 20 minutes.
- d. Report the spill to the laboratory's PI, who will report the spill to the Responsible Official if a select agent or toxin is involved.
- e. Soak up disinfectant and spill with paper towels. Drain catch basin into a container. Lift front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill.
- f. Autoclave all clean-up materials before disposal in the biohazard waste container.
- g. Wash hands and any exposed surfaces thoroughly after the clean-up procedure.

7. BSC Failure:

- a. **DO NOT use the BSC if the ALARM sounds** or if there are other indications of cabinet malfunction such as no airflow, HEPA filter failure, or unusual noises.
- b. Avoid personal contamination, close open containers of biohazardous material, surface-decontaminate, and remove them from the BSC.
- c. Surface-decontaminate and remove all other equipment/materials from the BSC.
- d. Remove and dispose of biohazardous waste.
- e. Remove any contaminated PPE and place in appropriate waste receptacle.
- f. Switch off the alarm and blower motor.
- g. Affix a warning sign (e.g., "OUT OF ORDER. DO NOT USE.") to the cabinet.
- h. If the failure is due to a temporary power outage, restart and decontaminate the BSC when the power returns.
- i. If the failure is due to BSC malfunction, close the sash and contact admin.tbep@utoronto.ca to have the cabinet serviced. Ensure that the BSC is decontaminated before any internal repairs are carried out.

- j. If users may have been exposed to infectious material due to cabinet failure, then the supervisor must be promptly notified, an incident report completed (<https://ehs.utoronto.ca/report-an-incident/>), and the appropriate first aid and medical follow-up action taken.

8. References:

- a) <https://ehs.utoronto.ca/wp-content/uploads/2016/10/Biosafety-Permit-Application-Form.pdf>
- b) <https://www.lsuhs.edu/admin/pfm/ehs/docs/bsc.pdf>
- c) <https://www.asu.edu/ehs/documents/asu-bsc-sop.pdf>
- d) <http://www.nipissingu.ca/departments/human-resources/health-and-safety/Lab-Safety/Documents/Nipissing%20University%20BioSafety%20Cabinet%20Use%20SOP.pdf>
- e) https://www.tru.ca/_shared/assets/Biological_Safety_Cabinet_Power_Failure39706.pdf