Class II Biosafety Cabinets

Proper Use of Biosafety Cabinet

A biosafety cabinet (BSC) is a valuable supplement to good sterile technique and a containment device when working with biohazardous agents/materials. It is advisable not to store too many items inside the cabinet; this would hinder the cabinet from performing at its full capacity and would compromise the sterility of materials inside the BSC, leading to contamination of handled samples, and also to personnel exposure to biohazardous materials. If a BSC is not operated correctly, it will not provide adequate protection. Below are some “Golden Rules” when working within a BSC to avoid contamination of samples and personnel exposure to the agents they are working with.

Follow these start-up procedures when preparing for work in a BSC:

**Plan your experiment.** Prepare a written checklist of the items that are needed for the experiment. Gather these materials before beginning the work, and be sure to include personal protective equipment (PPE) and waste disposal containers.

**Turn on the cabinet.** If the cabinet is not running, turn on the blower switch. Before using the BSC, let it run at least five minutes to purge airborne contaminants from the work area.

**Turn off ultraviolet (UV) light if in use and ensure that the sash is in the appropriate position.** UV radiation is hazardous to your health. Newer cabinets have a safety circuit that allows the UV light to activate only if the sash is completely closed. Do not adjust this important setting! An alarm sounds and flashes when the sash is opened too high or closed too low. The alarm can be silenced for five minutes at a time when loading large equipment and cleaning. Never work in the cabinet when the sash is not at the correct height.
Check certification date of the BSC. NSF International Standard/American National Standard for Biosafety Cabinetry - NSF/ANSI 49 - 2016 for Class II biosafety cabinetry establishes performance criteria and provides the minimum testing requirements that are accepted in the United States. Cabinets that meet the Standard and are certified by NSF bear an NSF mark. The operational integrity of a BSC must be validated before it is placed into service, after it has been repaired or relocated, and annually thereafter by qualified technicians (accredited field certifiers).

Check Magnehelic gauge. Magnehelic gauge measures the air pressure that air is pushed through the high-efficiency particulate air (HEPA) filter/ultra-low particulate air (ULPA) filter. The Magnehelic gauge should read close to the setting at the last certification (record that reading for easy reference). A reading that is too high or too low may indicate a problem with the airflows. Do not work in the cabinet if the Magnehelic gauge indicates a problem. Inform your PI / Laboratory Supervisor!

Confirm inward airflow. Hold a tissue at the middle of the edge of the viewing panel and ensure that it is drawn in.

Do not work in a BSC if a warning light or alarm is signaling.

PPE should be worn whenever using a BSC. Laboratory coat, gloves (pulled over the wrists of the lab coat, double gloving is recommended for some procedures), and safety glasses. Chemical splash goggles/masks may be required for some procedures.

Disinfect the work surface, interior walls (not including the supply diffuser), and the interior surface of the window. Use a disinfectant appropriate for the work being conducted in the laboratory: 70% alcohol (ethanol/isopropanol), a 1:100 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. Remove residual chlorine (could corrode the surfaces of BSC) by wiping the surfaces with 70% alcohol or sterile water. Check that the drain pan valve is closed before operation (if drain pan valve is present).

**Reminder:** Do not store supplies on top of the BSC. The HEPA/ULPA filter is located there, and it is easily damaged!

![Damaged HEPA filter on top of a BSC](image)

Follow these procedures for working in a BSC:

**Prepare to begin work.** Don protective clothing, safety glasses and gloves. Surface decontaminate all materials and containers with 70% alcohol before placing them into the cabinet. Deposit all items as far away from the sash as possible. Especially aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) should be placed toward the rear of the cabinet. All operations should be performed on the work surface at least four (4) inches from the inside edge of the front grill. Try to keep the work surface as neat as possible. Work may be performed on disinfectant-soaked absorbent towels to capture splatters and splashes. Avoid overloading the work area, thereby compromising the efficacy of the BSC. Limit items inside the cabinet to only the most essential. Be sure not to obstruct the front and rear air grilles. When untreated laboratory air is blocked from entering the front grille, it can flow over the work surface contaminating the material worked with and posing a risk to personnel. Keep disinfectant and paper towels handy for spills. Wait five (5) minutes after all materials have been placed in the BSC before beginning work. This time is needed to purge airborne contaminants from the work area.
Use only the items essential to your task in the safety cabinet. Anything placed into the cabinet becomes contaminated—pens, pencils, clipboards, etc. If this occurs, always disinfect the item before taking it out.

Work from clean to dirty. Organize supplies so that work can be segregated from the clean side of the cabinet to the dirty side. Avoid moving dirty items over clean ones to prevent cross-contamination of the experiment. Avoid excessive movement of hands and arms through the front access opening during use. When you do enter or exit the cabinet, do so from straight on and allow the cabinet to stabilize before resuming work. Keep discarded, contaminated materials to the rear of the cabinet. A small biohazard collection bag needs to be located inside BSC. Do not discard materials in containers outside of the cabinet. Always use mechanical pipetting aids. Traffic around the BSC should be minimized and drafts from doors and air conditioning should be avoided. If there is a spill during use, surface decontaminate all objects in the cabinet and disinfect the working area of the cabinet while it is still in operation (do not turn the cabinet off). 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.
A typical layout for working “clean to dirty” within a BSC

Collect waste materials. Workers using infectious materials should collect their wastes inside of the cabinet. Pipettes should be decontaminated within the cabinet. Disinfect items in a shallow pan filled with disinfectant. Other biohazard waste (plastic pipette tips, gloves, paper towels, etc.) should be similarly bagged inside the cabinet to avoid spreading contamination. Repeatedly moving arms in and out of the cabinet to deposit waste in a container outside of the BSC will compromise the airflow and containment provided by the BSC.

Avoid using open flames (Bunsen burner) inside BSCs. Flames disrupt the airflow and contribute to the heat load inside the BSC. Flames can burn holes in HEPA/ULPA filters. Flammable gasses (gasses are not captured by HEPA/ULPA filters) may recirculate and build up to the Lower Explosive Level (LEL) and cause an explosion or fire. If a flame is necessary, use a burner with a pilot light and place it to the rear of the workspace.
Follow these procedures upon completion of the work in a BSC:

**Surface-decontaminate objects / remove waste materials.** Wipe down all objects with disinfectant before removing them from the BSC. All items within BSC, including equipment, should be surface-decontaminated and removed from the cabinet when work is completed, since residual culture media may provide an opportunity for microbial growth. Removing waste from the BSC that is untreated or not packaged in a leak-proof container can spread contamination. Be sure to seal any bags and cover open containers before removing them from the cabinet.

*When removing arms from BSC, surface decontaminate gloves first and then move arms slowly out of the cabinet (in direction perpendicular to plane of work zone opening)!*

**Clean up.** After the cabinet has been emptied, wipe down the interior cabinet surfaces (work surface, sides, back, and interior of the window) with a disinfectant. 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. When the cabinet is shut off, ensure the sash is closed completely. Remove the work surface periodically and disinfect the area beneath it (including the catch pan).

**Remove contaminated gloves and dispose of them as appropriate.** Wash hands with soap and warm water.

**Turn on the UV light if BSC is equipped with one.** UV lights can accumulate dust and dirt that will block the germicidal effectiveness of the light. Bulbs can continue working for months or years after they stop providing the necessary wattage for disinfection purposes. The bulb should be wiped with alcohol weekly (while turned off), to remove dust. Additionally, UV light should be tested for efficacy to ensure that it is emitting a germicidal wavelength or bulb should be replaced annually. UV radiation should not take the place of
chemical disinfection of the cabinet interior. Make sure the sash is closed completely before using UV light. The glass sash will protect you from UV radiation.

**Common Errors to Avoid**

- Keep papers, paper towels, plastic backed absorbent toweling, vials, pipette wraps, or any other objects from being pulled into the back, front, or side slots of the BSC.
- Do not store equipment or supplies in the cabinet.
- Do not use the top of the cabinet for storage. The HEPA/ULPA filter could be damaged and the airflow disrupted.
- Do not place items on the front or rear perforated grills. This reduces the airflow.
- Do not rest arms on the front gill. This reduces airflow.
- Make sure the cabinet is level. If the cabinet is not, the airflow can be affected.
- Never disengage the alarm, as it indicates improper airflow, thereby affecting performance and endangering the researcher or the experiment.
- Never completely close the window sash with the motor running. This may cause the motor to burn out.