

Class II Biological Safety Cabinets (BSC) are very effective, primary containment devices designed to protect the operator from infectious agents. Additionally, BSCs protect experimental materials from outside contamination and prevent biohazardous materials from escaping into the environment.

SETTING UP THE BSC FOR WORK

- Verify that the BSC certification is up-to-date. Do NOT work in an uncertified BSC.
- Turn on BSC to purge work area for at least fifteen (15) minutes
- Ensure that the UV light is OFF while working.
 - ◇ Disinfect work surfaces, interior back and side walls, and interior window with 10% bleach, followed by sterile water, and finish with 70% ethanol.
- Load ONLY the NECESSARY materials and reagents as recommended in Figure 1.
 - ◇ Disinfect all items (e.g., equipment, outer surface of medium bottles) before placing them in the BSC.
 - ◇ Place materials 3-5 inches away from the grills.
 - ◇ Decontaminate serological pipettes by placing them in a shallow leak-proof pan or tall beaker filled with liquid disinfectant.

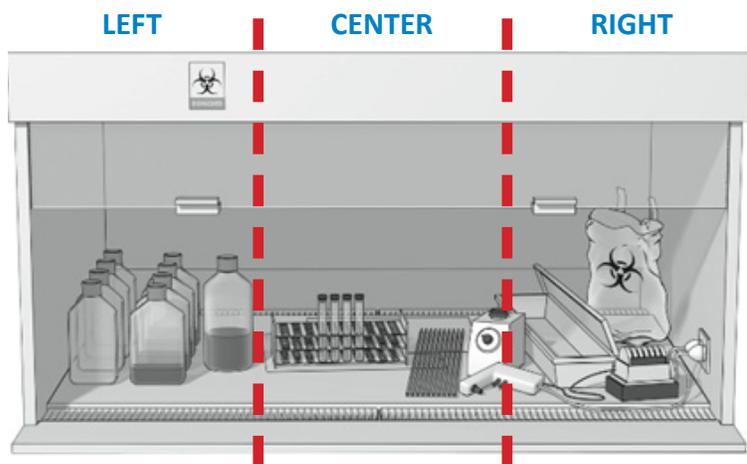


Figure 1. A typical layout for working “clean to dirty” within a Class II BSC (source BMBL 5th Edition).

Clean cultures (left-clean area) can be inoculated (center-work area); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right-dirty area). This arrangement is reversed for left-handed persons.

WHAT I NEED TO DO...

- Never store supplies on top of the BSC to avoid damage to the HEPA filter.
- Never use Bunsen burners or other open flames inside the BSC.
- Always decontaminate the BSC before and after use, and prior to being moved.
- Certify the BSC upon installation, annually, after repairs, and, after relocation. Ensure certification is always up-to-date.

WORKING IN THE BSC

- Ensure alarm is operational. Open sash to proper operating level.
- Always work from “clean to dirty”; this prevents cross-contamination.
- Use good microbiological practices (GMP) to avoid aerosol generation, splatter, or cross-contamination.
- Work towards back of BSC to allow contaminated air to exit through the back grill.
- Avoid activities that cause turbulence and disrupt the air flow pattern of the BSC such as:
 - ◇ Excessive hand movements inside the BSC.
 - ◇ Frequent movement in and out of the BSC.
 - ◇ Blocking front and/or back grills with equipment, paper, and/or supplies.
 - ◇ Personnel walking near or in the immediate vicinity of the BSC.
 - ◇ Open plates, tubes, and bottles at a slight angle and recap as soon as possible (avoid putting down caps or lids)
- Decontaminate pipette tips and pipettes by drawing disinfectant up inside them before discard. Discard pipette tips in small biohazard bag.

- If using a vacuum, ensure that a liquid disinfectant trap or commercial guardian canister trap with an in-line HEPA filter is in place to protect the building vacuum system as shown in Figures 2 and 3, respectively.

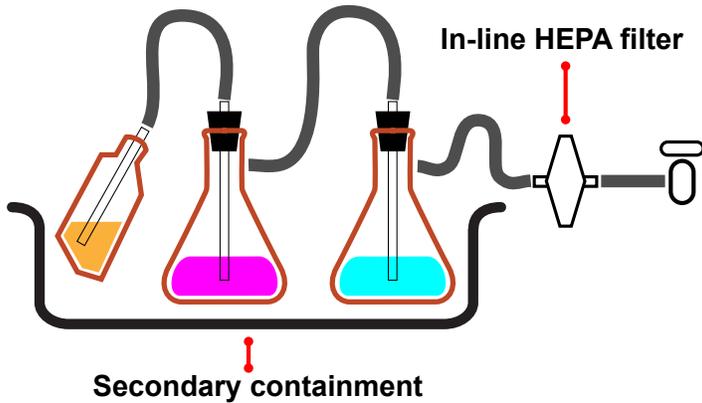


Figure 2. CDC/NIH-Liquid disinfectant trap with in-line HEPA filter

NOTE: Flasks must be placed in secondary containment and HEPA filters must be replaced as needed.



Figure 3. Commercial Guardian Canister System

AT THE END OF WORK IN THE BSC

- Wipe down culture plates, flasks, equipment, and supplies with disinfectant wipes prior to removing from the BSC.
- Disinfect gloves before removing hands from BSC.
- Place all solid and liquid waste in appropriate waste containers. Refer to the [Hazardous Waste Disposal Fact Sheet](#) for more information.
- When using a vacuum line, disinfect the line and the trap using a 1:10 dilution of household bleach. Empty the trap.

- Close or tie small biohazard bags with tape or a single knot.
 - ◊ Wipe down with disinfectant wipes.
 - ◊ Remove small bags from the BSC.
 - ◊ Place in the big biohazard waste container.
- Close or tie the biohazardous bag containing the serological pipettes.
 - ◊ Wipe down with disinfectant wipes.
 - ◊ Remove from the BSC.
 - ◊ Place in the big biohazard waste container.
- When the BSC is empty, disinfect work surfaces, interior walls, and window with appropriate disinfectant.
- Disinfect BSC trough and grill when a spill or contamination is noted.
- When shutting down the BSC, allow at least five (5) minutes to purge the interior prior to shutting it off.
- Turn off lights and close the window sash. Never completely close the window sash with the motor running.



Figure 4. Biohazard and sharps containers

REFERENCES

Selection, Installation, and Use of Biological Safety Cabinet, [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\) 5th Edition \(2009\)](#) - CDC/NIH

[Laboratory Biosafety Manual, Second Edition \(Revised\)](#) Interim guidelines (2003) - World Health Organization

USC Biosafety manual - <https://ehs.usc.edu/new-biosafety-manual/>