

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
- Disinfect work surfaces, interior back and side walls, and interior sash with 10% bleach, followed by sterile water, and finish with 70% ethanol.
- Load only the necessary materials and reagents needed for work into the BSC as recommended in Figure 1.
 - Disinfect all items (e.g., equipment, outer surface of medium bottles) before placing them in the BSC.
 - Place materials and equipment 3-5 inches away from the front and back grills.
 - Set up waste collection area - decontaminate serological pipettes by placing them in a shallow leak-proof pan or tall beaker containing 10% bleach for disinfecting.

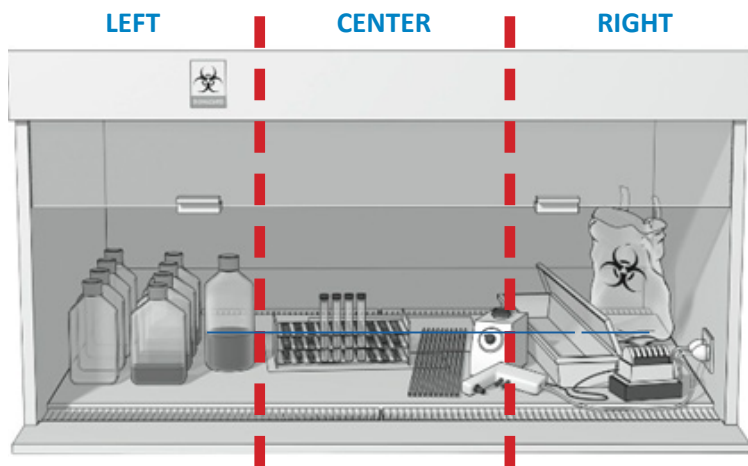


Figure 1. A typical layout for working “clean to dirty” within a Class II BSC (source BMBL 5th Edition).

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 or labeled beaker (right-dirty area) for disposal outside the BSC once work finished. This arrangement is reversed for left-handed persons.

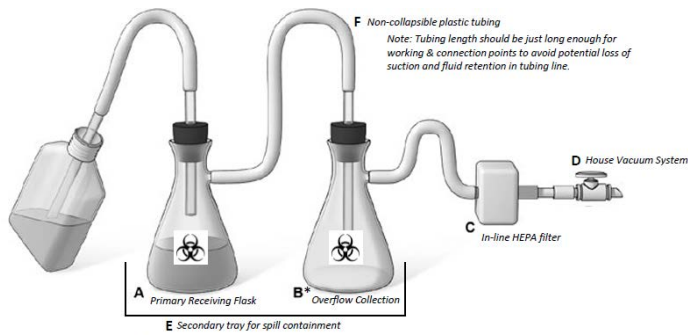
WHAT I NEED TO DO...

- Do not use the BSC as a long term storage space.
- 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.
- 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, between a 10 - 12 inch working gap.
- Always work from “clean to dirty”; this prevents cross-contamination (see Figure 1).
- 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. Including resting wrists on
 - 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).
- For waste - collect pipette tips, serological pipettes, and other solid waste inside the BSC using a tall beaker containing 10% bleach or in a small biohazard labeled beaker or bag for final disposal in RMW boxes when work is completed. If generating sharps, utilize a sharps container inside the BSC.

- If using a vacuum, ensure that an in-line HEPA filter is in place to protect the building vacuum system as shown in Figures 2 and 3.
- Collection flasks should contain 10% bleach.



A: Primary receiving flask used to collect fluids. If biohazardous, add biohazard sticker and undiluted bleach to 15% final volume prior to drain disposal (if applicable).
 B: Second suction flask serves as a fluid overflow collection vessel. *B is not required at Rutgers but is highly recommended.
 C: An in-line HEPA filter is used to protect the vacuum system (D)
 D: The vacuum system, protected by the in-line HEPA filter (C)
 E: Spill containment tray provides secondary containment for aspirator flasks.
 F: Flexible clear Tygon plastic non-collapsible tubing for connecting flasks; tubing through rubber stoppers should extend below the sidearm.

Figure 2. Rutgers Liquid disinfectant trap with in-line HEPA filter



Figure 3. Commercial Guardian Canister System

AT THE END OF WORK IN THE BSC

- Wipe down culture plates, flasks, equipment, and supplies with 10% bleach followed by distilled water or 70% Ethanol as appropriate prior to removing from the BSC.
- Place all solid waste in appropriate RMW waste containers.
 - No liquids should be disposed of in RMW boxes.
 - Sharp bins containing TC slides, glass pasture pipettes, and/or syringes with or without needles, are sealed and disposed in RMW boxes when 3/4 full.
- When using a vacuum line:
 - Disinfect the hose line using a final 1:10 dilution of bleach. Clear all residual liquid from hose lines.
 - Empty the trap when 3/4 full. Dilute to a final volume of 10% bleach, incubate at room temp for 10-15 minutes prior to drain disposal.

- When the BSC is empty- disinfect work surfaces, interior walls, and sash with 10% bleach, followed by distilled water, and finish with 70% ethanol. Do not use the BSC for storage of equipment or supplies.
- If a spill or potential contamination happens within the BSC - disinfect using 10% bleach soaked towels on-top of spill for 10-15 minutes followed by distilled water and 70% Ethanol as appropriate. Remove the BSC working surface to clean underneath as needed.
- Allow the blower to run for at least five (5) minutes to purge the interior of the cabinet prior to shutting down the BSC (as appropriate).
- 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, [Bio-safety in Microbiological and Biomedical Laboratories \(BMBL\) 6th Edition](#) (2020) - CDC/NIH

[Laboratory Biosafety Manual, 4th Edition \(Revised\)](#)(2020) - World Health Organization

Rutgers Biosafety Manual - ipo.rutgers.edu/rehs/bio-safety-guide