**Proper Use of Class II Biosafety Cabinets**

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.

### 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.

**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.
• If using a vacuum, ensure that a liquid disinfectant trap or commercial Guardian™ Suction 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.

![Diagram of liquid disinfectant trap with in-line HEPA filter and secondary containment](image)

**Secondary containment**

*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.

◊ Wipe down with disinfectant wipes.
◊ Remove from the BSC.

◊ Place in the big biohazard waste container.

◊ Remove contaminated serological pipettes from pan or tall beaker containing disinfectant liquid (10% bleach). Ensure that pipettes have had contact time for at least fifteen (15) minutes.

◊ Dispose of pipettes (glass or plastic) in sharps container. See Figure 4.

◊ Drain disinfectant liquid (10% bleach) in the sink. Do NOT dispose of other chemical liquids down the sink.

◊ Use disinfectant wipes to wipe down the pan or tall beaker.

• 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.

• 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.
  ◊ Disinfect BSC trough and grill when a spill or contamination is noted.

**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](https://ehs.usc.edu/new-biosafety-manual/) 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 from the BSC.

• Place in the big biohazard waste container.

• Remove contaminated serological pipettes from pan or tall beaker containing disinfectant liquid (10% bleach). Ensure that pipettes have had contact time for at least fifteen (15) minutes.

• Dispose of pipettes (glass or plastic) in sharps container. See Figure 4.

• Drain disinfectant liquid (10% bleach) in the sink. Do NOT dispose of other chemical liquids down the sink.

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• 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.

**REFERENCES**

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


- [USC Biosafety manual](https://ehs.usc.edu/new-biosafety-manual/)

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