TEXAS A&M UNIVERSITY SAN ANTONIO
Research and Academic Environmental Health & Safety

Guidelines: Biosafety Cabinet

☐ Process ☐ Hazardous Chemical ☐ Hazardous Class ☒ Equipment ☐ Experiment

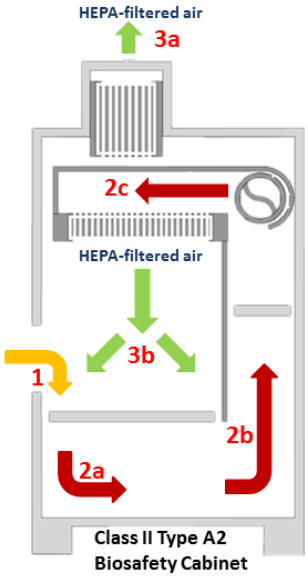
1.0 Introduction

Biological Safety Cabinets, (BSCs), also known as tissue culture hoods, are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Class II BSCs rely on directional movement of air to provide containment. Airflow is drawn into the front grille of the BSC, providing personnel protection. The most used BSC is a Class II A2. This type of BSC is not suitable for volatile solvents.

BSC installation, required annual certification, decontamination and maintenance **must** be done by certified (accredited by the National Sanitation Foundation) professionals.

2.0 How does a Biosafety Cabinet Work?

The Class II Type A2 biosafety cabinet is the most common cabinet on campus. It uses a curtain of air and HEPA filters to provide both containment and a sterile environment. Below is a schematic explaining how the cabinet functions:



Air Flow Pattern

1. Dirty room air is drawn into the front of the cabinet and mixes with contaminated air from the chamber.

2a. Contaminated air is pushed below the work surface

2b. Contaminated air is drawn up through the plenum.

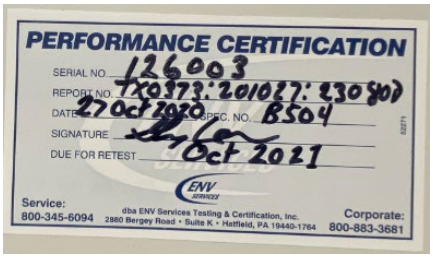
2c. Contaminated air is pushed through HEPA filters by cabinet blower motor.

3a. 30% of HEPA filtered air leaves through the top of cabinet and recirculates into room or is removed by canopy exhaust.

3b. 70% of HEPA filtered air enters the cabinet from above and flows down on work surfaces under unidirectional flow.

3.0 Preparing BSC for Work

1. Confirm BSC annual (within 12 months) certification is current; information found on sticker on front of BSC.
2. If the BSC has a UV light, turn the UV light off prior to starting work.
3. Operate the BSC blowers for at least 3-5 minutes before beginning work to allow the BSC to "purge" particulates. Listen for the blower to verify there is airflow.
4. If installed, verify the BSC's gauges and indicators have the appropriate reading.



Example of a Certification sticker found on BSC's

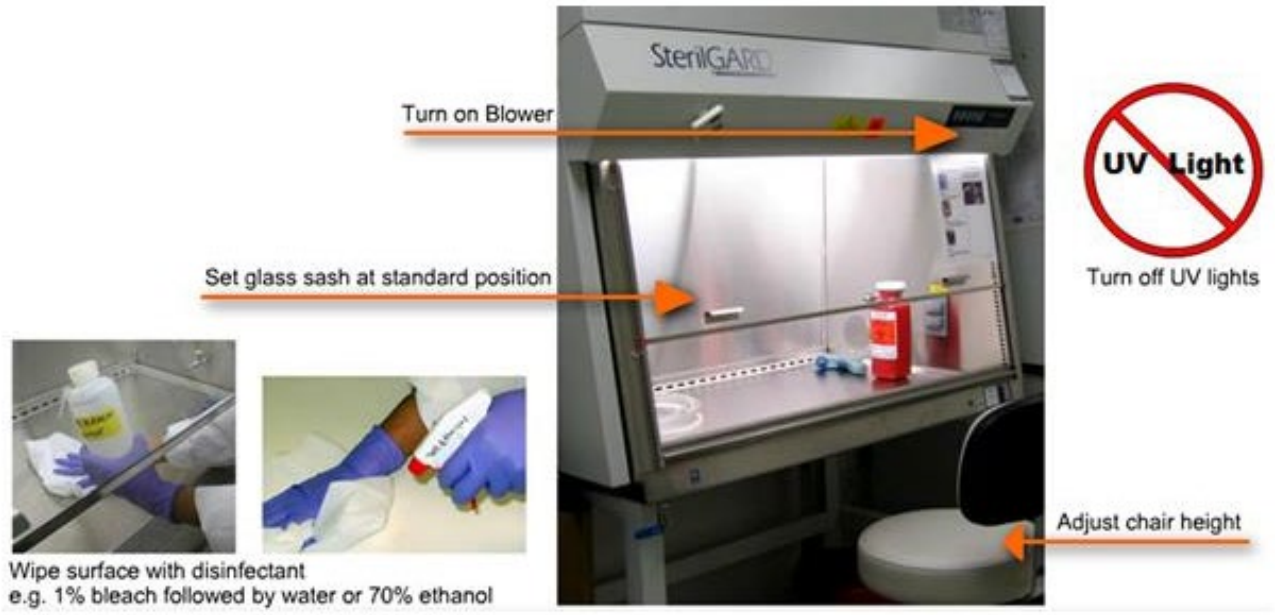
5. **WARNING**: Never operate a BSC while a warning light or alarm is on. Call RAEHS at (210) 784-2822 to service failing BSCs.
6. Disinfect all interior surfaces of BSC work area with an appropriate surface disinfectant (i.e. 10% bleach followed by 70% ethanol).

- Work surface
- Interior walls (except for the supply filter diffuser)
- Interior surface of the window

  NOTE: Use one the following disinfectants to wipe down the surfaces:
  - 70% ethanol (EtOH)
  - A 1:10 dilution of household bleach (i.e., 0.05% sodium hypochlorite)

    NOTE: When bleach is used, a second wiping with sterile water is needed to remove the residual chlorine, which may eventually corrode stainless steel surfaces.
- Other disinfectant as determined by the investigator to meet the requirements of the activity

7. Wipe down the surfaces of all materials and containers that will be placed in the cabinet with 70% EtOH to reduce the introduction of contaminants to the cabinet environment.
8. Store extra supplies (e.g., additional gloves, culture plates or flasks, culture media) outside the BSC



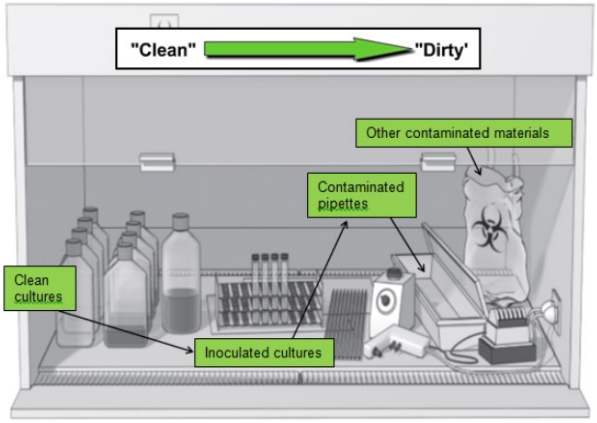
Prepare the BSC for Work. Source: Berkeley Lab EHS

9. Place all the equipment and supplies, including receptacle for waste and used pipettes, inside the BSC to minimize entering and exiting. When placing the equipment and supplies into the BSC:
   a. Segregate items that will remain clean from the ones that will become contaminated
   b. Place all materials as far back in the BSC as practical, toward the rear edge of the work surface and away from the front grille of the cabinet
   c. Place aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) toward the rear of the cabinet
   d. Place bulky items such as biohazard bags, discard pipette trays and vacuum collection flasks to one side of the interior of the cabinet
   e. Keep all materials at least four inches inside the sash opening. Never place items on the front or rear perforated grills.
10. Connect aspirator bottles to vacuum system (including an inline HEPA filter) or, alternatively, use a collection flask to suction liquid waste. Both systems must contain the appropriate disinfectant.
11. After placing equipment inside, close the sash to the proper operating height.
12. Wait 2-3 more minutes before beginning work.
13. Close drain valve

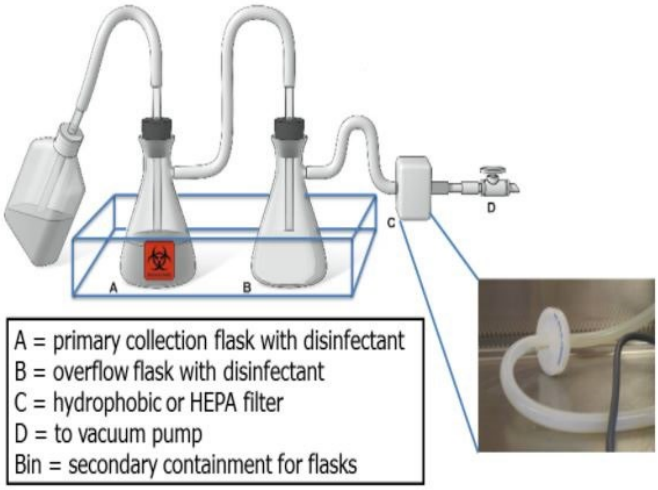
4.0 Working in the BSC

1. When working in the BSC, move arms in and out slowly, perpendicular to the face opening to reduce disruption of air curtain.
2. Perform all operations at least 4 inches from the front grille on the work surface.
3. Do not bring potentially contaminated materials out of the BSC until they have been surface decontaminated.
4. Disposable underpads can be placed on the work surface but **must** not cover the front or rear grille openings. The use of toweling facilitates routine cleanup and reduces splatter and aerosol generation during an overt spill.
5. Place all material as far back in the BSC as practical, toward the rear edge of the work surface and away from the front grille of the BSC.
6. Place aerosol-generating equipment (e.g. vortex mixers, tabletop centrifuges) toward the rear of the BSC.
7. Do not place any objects such as papers, notebooks, etc. on the front grille.
8. Loose or light weight items (i.e pipette sleeve, paper towel, Kimwipes) should not be left inside the BSC at any time to reduce the potential of being drawn into the containment/motor area of the BSC.
9. The workflow should be from “clean to dirty”. Materials and supplies should be placed in the BSC in such a way as to limit the movement of “dirty” items over “clean” ones.
10. Confirm everything necessary for the procedure is already inside the BSC and is sterile.
11. Ensure the vertical sliding sash is at the correct height.
12. Allow only one BSC operator at a time.
13. Sit at the BSC with armpits level with the bottom of the sash.
14. Minimize activities that create eddy currents (opening and closing doors and windows, personnel walking near the cabinet).
15. Perform all work using a limited number of slow movements, as quick movements disrupt the air barrier.
16. Hold Petri dishes and tissue culture plate lids above the open sterile surface to minimize direct impact of downward air.
17. Recap and cover bottles and tubes as soon as possible.
18. Decontaminate bacteriological loops and needles.

Typical Work Layout Inside a BSC. *Shown above is a typical layout for working “clean to dirty” within a Class II BSC. Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan, and other contaminated materials can be placed in the biohazard bag (right). This arrangement is reversed for left-handed people. Source: adapted from BMBL, fifth edition, Appendix A.*



5.0 Vacuum Line Protection



- Place a hydrophobic or HEPA filter before the vacuum line; filters are directional so make sure writing on the filter faces the overflow flask.
- Connect the primary flask to an overflow collection flask and to an in-line HEPA filter.
- Add an appropriate volume of disinfectant to primary flask to disinfect the final volume of liquid
- 1:10 (v/v) dilution of household bleach; bleach should be refreshed daily to ensure disinfecting effectiveness
- The vacuum flasks should be set up within the BSC.
- Place a biohazard sticker on the primary collection flask or the secondary container

Once inactivation occurs, liquid materials can be disposed of as noninfectious waste in the sink. Empty the waste from the flask when one of the following scenarios occurs:

- Daily
- Completion of process or experiment
- When the primary collection flask is ¾ full

6.0 After Using the BSC

1. Turn off equipment used to decontaminate bacteriologic loops and needles, if used.
2. Decontaminate materials that will be removed from the BSC. Refer to the laboratory SOP for instructions about the decontamination process within the laboratory.
3. Place contaminated items into a biohazard bag, discard tray, or other suitable container prior to removal from the BSC.
4. Run the BSC for three minutes, WITH NO ACTIVITY, so the airborne contaminates will be purged from the work area
5. Remove equipment.
6. Wipe the interior surfaces with 70% ethanol.

7.0 UV Lamps in a BSC

The CDC and the National Institutes of Health (NIH) agree that Ultraviolet (UV) lamps are not recommended nor required in BSC. UV lamps **must** be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer. Proper use and cleaning of BSC negates any need for the use of UV lamps. Numerous factors affect the activity of the germicidal effect of UV light, which require regular cleaning, maintenance and monitoring to ensure germicidal activity.