3.17. Biosafety Equipment

3.17.1. Biosafety Cabinets (BSCs)

The BSC is designed to provide protection to the product, the user, and the environment when appropriate practices and procedures are followed. Three types of BSCs (Class I, II, III) and the horizontal laminar flow cabinet are described below.

The common element to all classes of BSCs is the high efficiency particulate air (HEPA) filter. This filter removes particles of 0.3 microns with an efficiency of 99.97%. However, it does not remove vapors or gases.

The BSC requires regular maintenance and certification and should be completed by an NSF 49 accredited vendor to assure that it protects you, your experiments, and the environment. Each cabinet must be certified when it is installed, each time it is moved or repaired, and at least annually. Annual certification is a requirement under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (Appendix G-II-C-4-j of the Guidelines). Individual departments or PIs are responsible for costs of certification and repairs or replacement of HEPA filters. Annual certification is verified by IUEHS Biosafety during annual inspections and before IBC protocol approvals.

3.17.1.1. Types of Biosafety Cabinets

- **Class I BSCs** protect personnel and the environment, but not research materials. They provide an inward flow of unfiltered air, similar to a chemical fume hood, which protects the worker from the material in the cabinet. The environment is protected by HEPA filtration of the exhaust air before it is discharged into the laboratory or ducted outside via the building exhaust.

- **Class II BSCs** (Types A1, A2, B1, B2) provide personnel, environment, and product protection. Air is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air within the cabinet provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air passes through the exhaust HEPA filter, it is contaminant-free (environmental protection), and may be recirculated back into the laboratory (Type A) or ducted out of the building (Type B).

- **Class III BSCs** (sometimes called Class III glove boxes) were designed for work with infectious agents that require BSL-4 containment, and provide maximum protection to the environment and the worker. The cabinet is gas-tight with a non-opening view window, and has rubber gloves attached to ports in the cabinet that allow for manipulation of materials in the cabinet. Air is filtered through one HEPA filter as it enters the cabinet, and through 2 HEPA filters before it is

exhausted to the outdoors. This type of cabinet provides the highest level of product, environmental, and personnel protection.

3.17.1.2. Installing or Relocating Biosafety Cabinets

After installing or relocating a BSC, work may not begin until the BSC has been certified and tested by an outside vendor to ensure proper functionality. Care should be taken when deciding on the initial placement or relocation of a BSC. BSCs should be placed away from doorways and high traffic areas. They should also be placed away from heating and cooling vents to help maintain proper airflow within the cabinet.

Please contact IUEHS Biosafety for consultation on proper placement of a biological safety cabinet.

3.17.1.3. Disposal of Biosafety Cabinets

BSC must be space decontaminated before disposal. Contact IUEHS Biosafety prior to the decontamination and disposal of the cabinet.

3.17.1.4. Repairs of Biosafety Cabinets

Repairs may only be conducted by NSF-accredited technicians. If your BSC is in need of a repair, contact IUEHS Biosafety for your respective campus for assistance locating a repair technician.

3.17.1.5. Operation of Class II Biological Safety Cabinets

- Turn on cabinet fan 15 minutes before beginning work.
- Disinfect the cabinet work surface with 70% ethanol or other disinfectant.
- Place supplies in the cabinet. Locate container inside the cabinet for disposal of pipettes (Movement of hands in and out of the cabinet to discard pipettes into an outside container disrupts the air barrier that maintains sterility inside the cabinet.).
- Work as far to the back (beyond the air split) of the BSC work space as possible. Always use mechanical pipetting aids.
- Do not work in a BSC while a warning light or alarm is signaling.
- Locate liquid waste traps inside cabinet and use a hydrophobic filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container (such as a cardboard box) to prevent spilling. It is recommended that a secondary flask be utilized. See 3.17.6
- Wear gloves and laboratory coat when working within the biosafety cabinet
- Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper air flow and the level of protection provided. Also, keep the front and rear grilles clear.
- When work is completed, remove equipment and supplies from

the cabinet. Wipe the work area with 70% ethanol and allow cabinet to run for 15 minutes.

- Some BSCs are equipped with ultraviolet (UV) lights. However, if good procedures are followed, UV lights are not needed. If one is used, due to the limited penetrating ability of UV light the tube should be wiped with alcohol every two weeks, while turned off, to remove dust. UV radiation must not take the place of 70% ethanol for disinfection of the cabinet interior.
- The UV lamp must never be on while an operator is working in the cabinet.
- Minimize traffic around the BSC and avoid drafts from doors and air conditioning.
- Do not put your head inside the BSC. This compromises the sterility of the environment and, more importantly, could expose you to infectious pathogens.
- Do not tamper with the BSC or interfere with its designed function. It was engineered to operate optimally with no obstructions around the sash or grilles.
- Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current which prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence which disrupts the pattern of HEPA-filtered air supplied to the work surface. Therefore, the **use of open flames is strongly discouraged and gas burners are prohibited in in biosafety cabinets.** When deemed absolutely necessary, touch-plate micro-burners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops can also be used.

3.17.2. Clean Air Benches

Horizontal laminar low benches are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user, providing only product protection. They can be used for certain clean activities, such as dust-free assembly of sterile equipment or electronic devices. However, clean air benches are not a substitute for a BSC in research laboratories and use when handling BSL-2 cell culture materials or potentially infectious materials is inappropriate.

3.17.3. Centrifuge Containment

- Examine centrifuge tubes and bottles for cracks or stress marks before using

them.

- Never overfill centrifuge tubes since leakage may occur when tubes are filled to capacity. Fill centrifuge tubes no more than 3/4 full.
- Centrifuge safety buckets and sealed rotors protect against release of aerosols.

3.17.4. Protection of Vacuum Lines

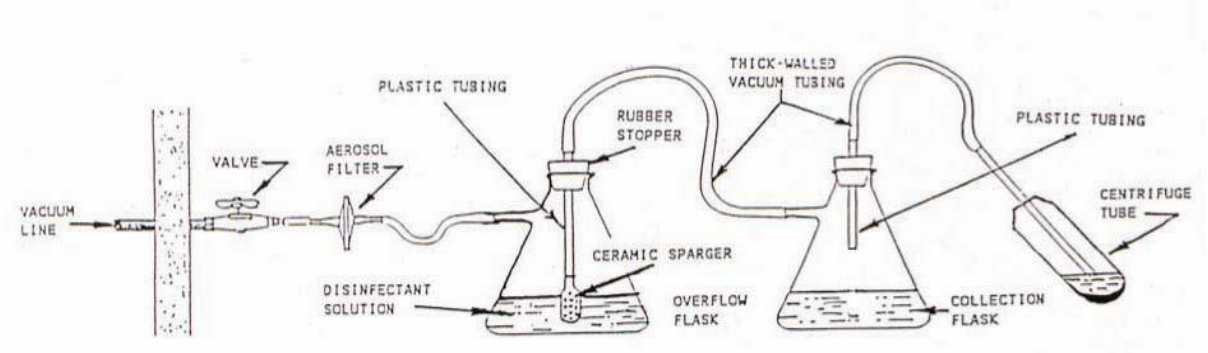
All central vacuum lines used to aspirate supernatants, tissue culture media, and other liquids that may contain microorganisms must be protected from contamination by the use of a collection flask and overflow flask. In addition a hydrophobic vacuum line filter must be used.

3.17.5. Collection and Overflow Flasks

- Collection tubes should extend at least 2 inches below the sidearm of the flask.
- Locate the collection flask inside the biosafety cabinet instead of on the floor, so the liquid level can be seen easily and the flask emptied before it overflows. The second flask (overflow) may be located outside the cabinet.
- If a glass flask is used at floor level, place it in a sturdy cardboard box or plastic container to prevent breakage by accidental kicking.
- In BSL-2 and BSL-3 laboratories, the use of Nalgene flasks is recommended to reduce the risk of breakage.

3.17.6. Vacuum Line Filter

A hydrophobic filter will prevent fluid and aerosol contamination of central vacuum systems or vacuum pumps. The filter will also prevent microorganisms from being exhausted by a vacuum pump into the environment. Hydrophobic filters such as the Whatman HEPA-Vent Filter are available from several scientific supply companies (Fisher Scientific, catalog #09-744-79).



An alternative to this setup is a medical grade suction canister, which is an increasingly popular option.

It can be found at:
http://extww02a.cardinal.com/us/en/distributedproducts/ASP/65651-212.asp?cat=surgerycenter