



Working Safely in the Biosafety Cabinet

The biosafety cabinet (BSC) is an essential piece of safety equipment for many biological laboratories. The BSC, an engineering control, is designed to provide personnel and environmental protection from potentially infectious materials and human pathogens.

BSCs are manufactured in three different classes (Class I, II and III). The common element in all biosafety cabinets is the high efficiency particulate air (HEPA) filter. HEPA filters can remove particles down to 0.3 microns with 99.97% efficiency and will trap most bacteria, viruses and spores. Vapors and gasses will not be captured by a HEPA filter.

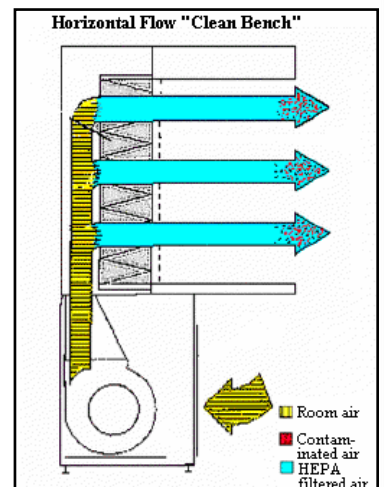
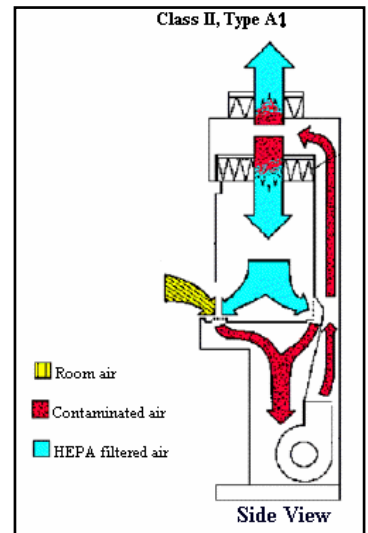
CLASS I BIOSAFETY CABINETS provide personnel and environmental protection, but do not provide a sterile work surface. Air flow in a Class I BSC is similar to that of a chemical fume hood, with air being drawn away from the worker and across the work surface. A HEPA filter on the cabinet exhaust protects the environment. Class I cabinets are useful for work that requires containment but not product sterility.

CLASS II BIOSAFETY CABINETS provide personnel, product, and environmental protection. Air is drawn around the worker into the front grill of the BSC, providing operator protection. HEPA filtered sterile air flows down onto the work surface, minimizing the potential for cross-contamination. Exhaust air passes through another HEPA filter before being released into the environment. Class II units are divided into four types: A1, A2, B1, and B2.

- ♦ **Type A1** units exhaust 30% of their air and recirculate 70% through HEPA filters. Type A1 units exhaust sterile air to the room atmosphere.
- ♦ **Type A2** units also exhaust 30% and recirculate 70% of their air through HEPA filters. HEPA filtered exhaust from type A2 BSCs may vent to the room atmosphere, or may be connected to the building exhaust system.
- ♦ **Type B1** cabinets exhaust 60% of their air and recirculate 40%. Exhaust air must be processed through a dedicated facility exhaust duct that leads to the roof.
- ♦ **Type B2** cabinets exhaust 100% of their air. No air is recirculated in a type B2 unit. Exhaust air must be ducted to a dedicated facility exhaust channel.

CLASS III BIOSAFETY CABINETS are designed to provide maximum protection to the worker and the environment. Sometimes called Class III glove boxes, these units are gas-tight enclosures with a non-opening view window. Intake air is filtered through a HEPA filter, and exhaust air passes through two HEPA filters before being exhausted to the outdoors.

HORIZONTAL LAMINAR FLOW CLEAN BENCHES are not biosafety cabinets. These devices provide product protection, but *do not protect the worker* or the environment. HEPA filtered air is discharged across the work surface and toward the user, effectively blowing any pathogens or contaminants into the user's breathing zone. *These units are not a substitute for a biosafety cabinet, and should never be used when working with pathogens.* They may be used for pouring media or other clean applications. EOHSS does not recommend the use of horizontal laminar flow clean benches for work with biological agents.

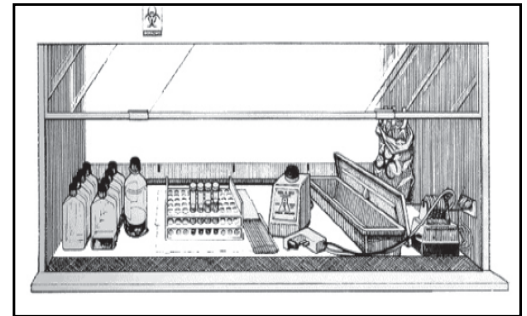




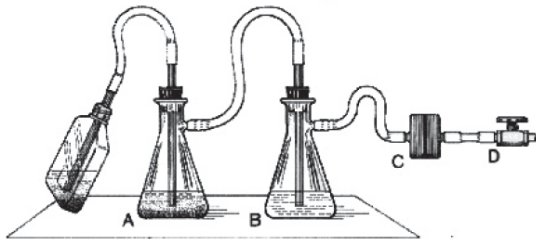
Working in the Biosafety Cabinet

Workers should follow this procedure when preparing to work in a BSC:

1. **Plan your experiment.** Prepare a written checklist of the items you will need for your experiment. Gather these materials before you begin working, and be sure to include your personal protective equipment and waste disposal containers.
2. **Turn on the cabinet.** If the cabinet is not running when you wish to begin work, turn on the power and let the cabinet run for at least 15 minutes. This cycle will purge the work area of any particles before you begin working.
3. **Disinfect the work surface.** Use a disinfectant appropriate for the work being conducted in your lab. If using a disinfectant such as bleach, wescodyne, vesphene, or another commercial preparation, it is helpful to follow up your wipe with a 70% ethanol rinse. Ethanol can help the disinfectant to evaporate cleanly.
4. **Prepare to begin work.** Line the work surface with plastic backed absorbent towels, or work over a tray. Place your supplies into the cabinet, as far away from the sash as possible. All operations should be performed on the work surface at least four (4) inches from the inside edge of the front grille. Try to keep the work surface as neat as possible.
5. **Work from clean to dirty.** Organize your supplies so that you can segregate your work from the clean side of the cabinet to the dirty side. Avoid moving dirty items over clean ones to prevent cross-contamination of your experiment.
6. **Protect vacuum lines.** If you will be using a vacuum, be sure to use a HEPA filter and inline disinfectant flasks to protect your vacuum system from contamination. The in-line flasks will catch any overflow, and the HEPA filter will prevent aerosols and particles from entering the vacuum line. Whatman Vacu-Guard Filters are available through UMDNJ General Stores.



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).



For aspiration, use a vacuum flask (A), with a second vacuum flask (B) connected as an overflow. Attach a HEPA filter capsule (C) to the second vacuum flask so that contaminants are not drawn into the vacuum system (D).

Source: <http://www.cdc.gov/od/ohs/pdffiles/BSC-3.pdf>

7. **Collect waste materials.** Workers using infectious materials should collect their wastes inside of the cabinet. Repeatedly moving arms in and out of the cabinet to deposit waste in a container outside of the BSC will compromise the air flow and containment provided by the BSC. Removing waste from the BSC that is untreated or not packaged in a leak-proof container can also spread contamination. Be sure to seal any bags and cover open containers before removing them from the cabinet.

8. **Clean up.** Wipe down all materials with disinfectant before removing from the BSC. After the cabinet is emptied, wipe down the interior cabinet surfaces with disinfectant. Allow the BSC to run for 15 minutes before turning it off.



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Common Errors to Avoid

- ♦ Keep the front and rear grills clear. Covering them with supplies or paper will compromise the cabinet's airflow.
- ♦ Do not store supplies on top of the BSC. The HEPA filter is located there, and it is easily damaged.
- ♦ Do not use an open flame in the BSC. It can create turbulence that disrupts air flow. If the gas to a Bunsen burner is accidentally turned on without the burner being lit, the concentration of gas will build up due to recirculation of cabinet air. The fan motor can act as an ignition source resulting in a serious explosion and fire. Use sterile disposable supplies or an electric bacticinerator instead. If an open flame is unavoidable, use a Touch-O-Matic Bunsen burner equipped with a microburner pilot light. The Touch-O-Matic provides flame on demand.
- ♦ Avoid moving your arms in and out of the cabinet during your experiment, and try to minimize activities that can cause eddy currents (opening doors, personnel walking near cabinet, etc.). Small pockets of turbulence can compromise air circulation in the BSC.



Bacticinerator
www1.fishersci.com



Touch-O-Matic
<http://canadawide.ca>

Certification and Repairs

Biosafety cabinets require regular certification and maintenance. All units must be certified on an annual basis by a professional technician. BSCs must also be certified when installed, moved, or repaired. In coordination with ANSI, the National Sanitation Foundation (NSF) maintains a standard for evaluation of class II cabinets. NSF also accredits BSC certification technicians.

If you are planning to move or dispose of a biosafety cabinet, it must be decontaminated by a professional cabinet service provider. The decontamination process will include treatment of the work surface, plenums and HEPA filters to render the unit safe for disposal.

RWJMS Shared Equipment Services (732/235-4455) arranges for annual certification, filter changes and repairs for RWJMS facilities. In other facilities, each laboratory is responsible for maintaining current certifications for their BSCs. Contact EOHSS if you would like more information about BSC certification companies servicing your campus.

References:

1. University of Maryland Biological Safety Cabinets Site:
<http://www.des.umd.edu/biosafety/rest/cabinets.html>
2. University of Michigan Helpful Hints on the Use of Biological Safety Cabinets:
<http://www.oseh.umich.edu//cabinet.html>
3. Stony Brook University Biosafety Cabinet Work Practices and Procedures:
<http://www.sunysb.edu/ehs/lab/biocabinets.shtml>
4. Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets:
<http://www.cdc.gov/od/ohs/pdffiles/BSC-3.pdf>

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