

SOP BIO-010 USE AND DECONTAMINATION OF THE BIOSAFETY CABINET

SCOPE

The purpose of this SOP is to demonstrate how to safely use and maintain the biological safety cabinets (BSC); and to ensure the proper containment of manipulated biological material as well as the safety of anyone operating the BSC.

DEFINITIONS

Biosafety cabinets (BSCs) are hoods with high efficiency particulate air (*HEPA*) filters that provide personnel, environmental, and product protection when appropriate practices and procedures are followed. Safety equipment including BSCs, PPE, or other physical containment devices (e.g. safety centrifuge cups) must be used whenever procedures with a potential to create infectious aerosols or splashes are conducted, or whenever high concentrations or large volumes of infectious agents are used. Examples of such procedures include pipetting, centrifuging, grinding, blending, shaking, mixing, vortexing, sonicating, opening containers with pressure differentials, or harvesting infected tissues. The BSC is the principal Biosafety level 2 (BL-2) device used in laboratories to provide such containment.

Three types of BSCs (Class I, II, and III) are used in microbiological laboratories. Open-fronted Class I and Class II BSCs are partial containment devices, which provide a primary barrier offering significant levels of protection to laboratory personnel and to the environment when used in combination with good microbiological techniques.

The **Class I BSC** is suitable for work involving low to moderate risk agents, where there is a need for containment but not for product protection. It provides protection to personnel and the environment from contaminants within the cabinet. The Class I BSC does not protect the product from "dirty" room air.

The **Class II BSC** meets requirements to protect personnel, the environment and the product since it protects the material inside the cabinet (e.g., cell cultures, microbiological stocks) from external contamination.

There are different types of Class II BSCs: Type A (A1, A2), Type B1 and Type B2. The major differences between these types are in the percent of air that is exhausted or recirculated, and the manner in which exhaust air is removed from the work area. Type B1 and B2 are BSCs ducted that can exhaust the air removed outside the laboratory area, outside the facility.

Although B1 is ducted, 40% of the air is recirculated and 60% removed or exhausted. BSCs Class II Type B2 are mostly ducted with 100% of the air exhausted

The gas-tight **Class III BSC** or glove box provides the highest attainable level of protection to personnel, the environment and the product. It is the only cabinet that provides a total physical barrier between the product and personnel. It is for use with high-risk biological agents and is used when absolute containment of highly infectious or hazardous material is required.

Additional information on the proper use and selection of a BSC can be found on 5th Ed Biosafety in Microbiological and Biomedical Laboratories (BMBL)¹.

Decontamination is the destruction of microorganisms to some safe level (but not necessarily zero).

Disinfection is the chemical or physical treatment that destroys most vegetative microbes, but not spores.

High Efficiency Particulate Air (HEPA) Filter traps 99.97% of particles of 0.3 µm in diameter and 99.99% of particles of greater or smaller size. The filter captures all infectious agents and ensuring that only clean air, free of microbes, is exhausted from the cabinet or directed to the work-surface.

Sanitization is the reduction of a microbial load on a surface to a safe public health level.

Sterilization is the total destruction of all microorganisms.

RESPONSIBILITIES:

Principal Investigator or Designated Person in Charge is responsible for providing all lab members adequate training to operate the BSC in a safety manner and ensures that the BSC properly maintained and annually re-certified.

Users, prior to operating the BSC, need to have training and demonstrate understanding of the proper work with BSC. All users must report any spills or accidents to EEM-EHS.

PROCEDURES:

Start of BSC Operation:

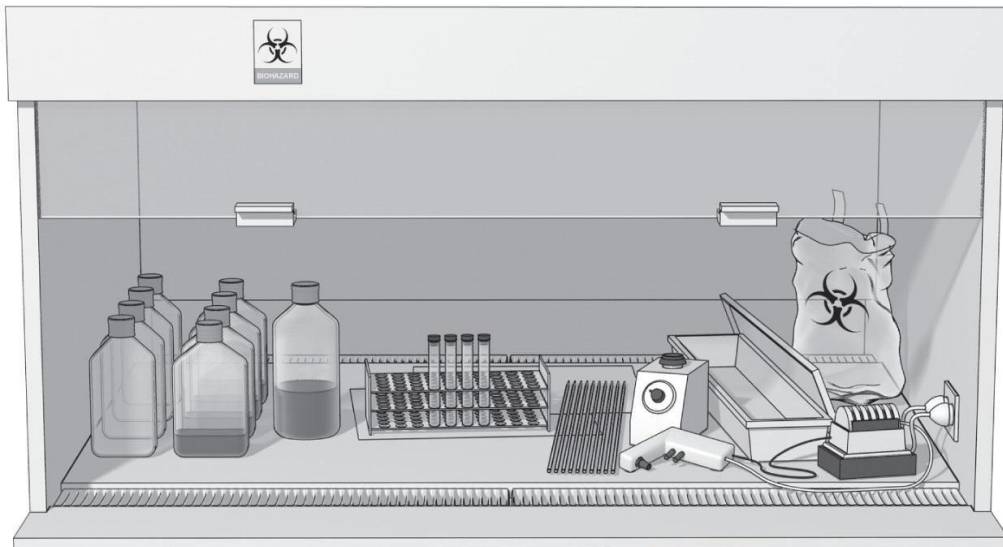
1. Wear appropriate PPE (lab coat, gloves with long cuffs, eye safety glasses if necessary and no open toed shoes in the lab; appropriate PPE will vary according to the type of work being performed);
2. Have all your supplies ready;
3. Turn on the BSC for at least 10 minutes (this allows adequate aeration of the cabinet);

¹ <http://www.cdc.gov/biosafety/publications/bmb15/>

4. Disinfect cabinet surfaces using an approved disinfectant such as 70% ethanol;
5. Do not rely upon ultraviolet (UV) light as the sole decontaminating agent. UV light loses its effectiveness over time and too often is not replaced before its intensity drops below optimal level. Even when the UV light is operating correctly, surface decontamination should be performed before and after every cabinet use
6. Start organizing your cabinet area, make sure to surface disinfect anything that you are bringing in and out of the cabinet;
7. Waste containers, suction collecting flasks, and dirty supplies beakers must be placed on one side of the cabinet. Avoid clutter as it will impede the laminar air flow;
8. Only bring in the necessary instruments and supplies;
9. Heat sources such as Bunsen burners are **not allowed** within the BSC;
10. Do not block the grill/grid area of the cabinet, this will allow adequate airflow and filtration. If you block the grid area, air will penetrate the lab space instead of being drawn towards the HEPA filter;
11. Your work should be done about one full palm-in (5-6 inches) away from the front grid.

Clean to Dirty Layout

The following graphic shows a typical layout “clean to dirty”² to work in a class II BSC. Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials (as pipettes-tips and small plastic tubes) can be placed in the small biohazard bag. This arrangement can be reversed for left-handed persons.



Left Side: Clean Cultures

Right Side: Waste and Dirty Supply Collection

² The clean to dirty layout was obtained from BMBL 5th Ed. Appendix A, figure 11 (2009) at <http://www.cdc.gov/biosafety/publications/bmbl5/>

End of BSC Operation

1. Take everything out accordingly, while disinfecting once again;
2. Once empty (do not store anything in BSC) disinfect the interior completely: that includes back and side walls, ceiling, inside bottom, and the inside of glass door shaft;
3. Let the BSC run for an additional 10-15 min after cleaning;
4. Check below the grid area to ensure no visible particles or spills were trapped;
5. If a UV light is being used it must be checked on a weekly basis (cleaned with lint free material soaked in alcohol), and certified on an annual basis to ensure that the proper wavelength for decontamination is being attained.

BSC Decontamination when working with Group Risk 2 Infectious Agents

In case of spills involving large amounts of infectious microorganism that overflow below the grid area, it will be necessary to have a complete decontamination that could involve the use of chemical or gases.

The decontamination by any chemical (Formaldehyde, Hydrogen Peroxide, and others) method requires the intervention of personnel trained for this procedure. The EEM-EHS department uses authorized vendors, and the Biosafety Officer coordinates the process with them. In some cases, decontamination with chemical gases can take 12-15 hours.

Certification/Recertification of BSC:

At UMass Lowell, the EEM-EHS Department oversees and manages the maintenance of biosafety cabinets and offers testing and certification for all of them. This service is done yearly during spring and it is offered free of charge to investigators or Departments.

To ensure proper work Biosafety cabinets, authorized technical personnel must test and recertify BSCs. Certification/re-certification should be done at least once a year in the following situations:

- Before initial use;
- After being moved from one location to another;
- After changing the HEPA filter;
- After cleaning/decontamination of a serious spill inside the cabinet.

To coordinate the disinfection by an authorized vendor or for additional information on decontamination, re-certification, spills, biological waste disposal and/or any biosafety issues, contact the Biosafety Officer at biosafety@uml.edu or call EEM-EHS at 978-934-2618.