UNIVERSITY OF
South Carolina

BIOLOGICAL SAFETY CABINET PROGRAM MANUAL

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1. INTRODUCTION

Biological safety cabinets (BSCs) are used at the University of South Carolina as a primary means of containment for working safely with infectious microorganisms. They act as primary barriers to prevent the escape of biological aerosols into the laboratory environment. This is an important function, because many laboratory techniques (e.g. pipetting, vortexing, sonicating) are known to produce inadvertent aerosols that can be readily inhaled by the laboratory worker. Proper maintenance of cabinets used for work at all biosafety levels cannot be over emphasized. A BSC must be routinely inspected and tested by training personnel, following strict protocols, to verify that it is working properly. BSCs are only one part of an overall biosafety program which requires consistent use of good microbiological practices, appropriate containment equipment, and proper facility design.

BSCs are designed, in varying combinations, for:

- Personnel Protection: Protects personnel from harmful agents inside the BSC.
- Product Protection: Protects the work, product, experiment or procedure performed in the BSC from contaminants in the lab environment or from cross contamination in the cabinet.
- Environmental Protection: Protects the environment from contaminants inside the BSC. There are three broad types of BSCs, each with varying degrees of protection:
  - Class I BSCs provide personnel and environmental protection, but not product protection. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. Class I BSCs are often used specifically for purposes that do not require product protection, such as to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or for procedures with potential to generate aerosols (e.g., cage dumping, culture aeration or tissue homogenization).
  - Class II BSCs provide personnel, environmental and product protection. Airflow is drawn into the front grille of the cabinet, providing personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination across the work surface of the cabinet. Class II BSCs are appropriate for work with agents assigned to biosafety levels 1-3. Class II BSCs provide the microbe-free work environment necessary for cell culture propagation and also may be used for the formulation of nonvolatile antineoplastic or chemotherapeutic drugs. However, this type of BSC must not be used for work involving volatile chemicals or gases. The Class II BSC is the most common type of BSC used at USC.
  - Class III BSCs are designed for work with highly infectious microbiological agents and provides maximum protection for the environment and the worker. It is a gas-tight enclosure with a non- opening view window. Both supply and exhaust air are HEPA filtered and these cabinets are not exhausted through the general lab exhaust system.

2. PURPOSE AND SCOPE

The purpose of the BSC Program Manual is to establish University policy for the selection, use, maintenance and certification of biological safety cabinets in USC laboratories. This manual has also been reviewed and approved by the USC Institutional Biosafety Committee (IBC).

3. SELECTION AND INSTALLATION

The Class II, Type A2 BSC is the most used cabinet in the USC research labs. This type of BSC provides personnel, product and environmental protection from hazardous particulates such as biological agents that require containment at Biosafety Level 1, 2 or 3. HEPA filtered exhaust air from a Class II, Type A2 BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations.

BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. This would allow for 100% of the filtered exhaust air to be discharged out of the laboratory. Provisions to assure proper safety cabinet performance and air system operation must be verified. The expense for installation and maintenance of a total-exhaust BSC is much higher, and therefore should only be selected and installed when justified based on a risk assessment of the research conducted in the lab (e.g., cell culture work with infectious agents and volatile toxic chemicals). The USC Biological Safety Officer must be notified for further guidance prior to ordering and installing a total-exhaust cabinet (i.e., Class II, Type B2).

Utility services needed within a BSC must be planned carefully. Protection of vacuum systems must be addressed. Electrical outlets inside the cabinet must be protected by ground fault circuit interrupters and should be supplied by an independent circuit. When propane or natural gas is provided, a clearly marked emergency gas shut-off valve outside the cabinet must be installed for fire safety. The use of compressed air within a BSC must be carefully considered and controlled to prevent aerosol production and reduce the potential for vessel pressurization.

Certain considerations must be met to ensure the maximum effectiveness of biological safety cabinets as primary barriers used during manipulations of infectious microorganisms. Whenever possible, adequate clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance and to ensure that the cabinet air re-circulated to the laboratory is not hindered. A 12-to-14-inch clearance above the cabinet may be required to provide for accurate air velocity measurement across the exhaust filter surface and for exhaust filter changes. BSCs should be located away from doors, windows that can be opened, and heavily traveled laboratory areas. The ideal location for the biological safety cabinet is remote from the entry (i.e., the rear of the laboratory away from traffic), since people walking parallel to the face of a BSC can disrupt the air curtain. Open windows, air supply registers, portable fans or laboratory equipment that creates air movement (e.g., centrifuges, vacuum pumps) should not be located near the BSC. Similarly, chemical fume hoods must not be located close to BSCs.

Proper selection and installation of BSCs must be in accordance with the most recent edition of *Primary Containment of Biohazards: Selection, Installation and Use of Biological Safety Cabinets* (available online at https://www.cdc.gov/labs/BMBL.html). This resource is published jointly by the U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health.

4. USE OF ULTRAVIOLET (UV) LAMPS

According to the CDC/NIH – "Ultraviolet (UV) lamps are not recommended in BSCs nor are they necessary. If installed, UV lamps must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the ultraviolet light. The lamps should be checked weekly with a UV meter to ensure that the appropriate intensity of UV light is being emitted. 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. If the cabinet has a sliding sash, close the sash when operating the UV lamp."

Some BSC manufacturers no longer include UV lamps in their Class II BSCs, unless specifically requested by the customer. The use of UV lamps can sometimes result in a false perception about their effectiveness. The ESCO Global has published a technical paper titled *UV Lamps in Laminar Flow and Biological Safety Cabinets* that is available for reference online at http://www.escoglobal.com/resources/pdf/white-papers/UV_lamps.pdf.

5. USE OF BIOLOGICAL SAFETY CABINETS

A properly certified and operational biological safety cabinet is an effective engineering control which must be used in concert with appropriate work practices, procedures, and other administrative controls to reduce the risk of exposure to infectious microorganisms. The following work practices and procedures should be in place when working in a BSC.

(1) Preparing for Work in a Class II BSC

- Place necessary materials in the BSC before beginning work to minimize the number and extent of air curtain disruptions compromising the fragile air barrier of the cabinet. Moving arms in and out slowly, perpendicular to the face opening of the cabinet will reduce the risk. Other personnel activities in the room (e.g., rapid movements near the BSC face, walking traffic, open/closing doors) may also disrupt the cabinet air barrier.

- Lab coats should be worn buttoned over street clothing; latex, vinyl, nitrile, or other suitable gloves are worn to provide hand protection.

- Before beginning work, the investigator should adjust the stool/chair height so that his/her face is above the front opening.

- The front grille must not be blocked with toweling, research notes, discarded plastic wrappers, pipetting devices, or other materials. All operations should be performed on the work surface at least four inches in from the front grille.

- Extra supplies should be stored outside the cabinet. Only the materials and equipment required for the immediate work should be placed in the BSC.

- BSCs are designed for 24-hour per day operation. Some researchers prefer continuous operation to help control the laboratory's level of dust and other airborne particulates.

- If the cabinet has been shut down, the blowers should be operated at least four minutes before beginning work to allow the cabinet to "purge".

- The work surface, the interior walls, and the interior surface of the window should be wiped with 70% ethanol (EtOH) or other appropriate disinfectant.
- The surfaces of all materials and containers placed into the cabinet should be wiped with 70% ethanol to reduce the introduction of contaminants into the cabinet.

(2) Placement of Materials In the BSC

- All materials should be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet.
- The biohazard collection bag should not be taped to the outside of the cabinet. Upright pipette collection containers should not be used in BSCs nor placed on the floor outside the cabinet. The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. Only horizontal pipette discard trays containing a chemical disinfectant should be used within the cabinet.
- Potentially contaminated materials should not be brought out of the cabinet until they have been surface decontaminated.

(3) Operations in a Class II BSC

- 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.
- Opened tubes or bottles should not be held in a vertical position. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impaction of downward air. Items should be recapped or covered as soon as possible.
- Open flames are not required in the near microbe-free environment of a biological safety cabinet.
- Aspirator bottles or suction flasks should be connected to an overflow collection flask containing disinfectant, and to an in-line HEPA or equivalent filter. This combination will provide protection to the central building vacuum system or vacuum pump.
- Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to inactivate the microorganisms as they are collected.
- Contaminated items should be placed into a biohazard bag, discard tray, or other suitable container prior to removal from the BSC.

(4) Cabinet Surface Decontamination

- With the cabinet blower running, all containers and equipment should be surface decontaminated and removed from the cabinet when work is completed.
- Investigators should remove their gloves and gowns in a manner to prevent contamination of unprotected skin and aerosol generation and wash their hands as the final step in safe microbiological practices.

6. CERTIFICATION OF BIOLOGICAL SAFETY CABINETS

(1) General Certification Requirements:

(a) All Class II BSCs must be tested and certified at least annually to ensure continued proper operation.

(b) All Class II BSCs will be tested and certified in accordance with specifications in NSF/ANSI Standard 49 Annex N-5 and the manufacturer’s specifications.

(c) The operational integrity must be validated by certification before a newly installed BSC is used and after a BSC has been repaired or relocated.

(d) After a BSC has been certified, a label will be prominently affixed to the front of the BSC, displaying the date of certification and name of the certifier.

(e) A copy of certification report will be provided to the laboratory staff.

(f) Only NSF accredited field certifiers will be used to test and certify BSCs.

(2) Performance Testing Requirements for Class II BSCs. Class II BSCs are the primary containment devices that protect the worker, product, and environment from exposure to microbiological agents. BSC operation, as specified by NSF/ANSI Standard 49 Annex N-5 needs to be verified at the time of installation and, as a minimum, annually thereafter. The purpose and acceptance level of the operational tests ensure the balance of inflow and exhaust air, the distribution of air onto the work surface, and the integrity of the cabinet and the filters. Other tests check electrical and physical features of the BSC. All Class II BSCs will be tested and certified as per NSF/ANSI 49 – 2020 specifications by using the following tests:

- Downflow Velocity Profile Test
- Inflow Velocity Test
- Airflow Smoke Patterns Test
- HEPA Filter Leak Test
- Cabinet Integrity Test (A1 Cabinets only)
- Electrical Leakage and Ground Circuit Resistance and Polarity Tests
- Lighting Intensity Test
- Vibration Test
- Noise Level Test
- UV Lamp Test

(3) Gas Decontamination (Reference NSF/ANSI 49 Annex I-2)

(a) BSCs that have been used for work involving infectious materials must be decontaminated before HEPA filters are changed or internal repair work is done.

(b) BSCs must be decontaminated prior to decommissioning and salvaging.

(c) Before a BSC is relocated, a risk assessment considering the agents manipulated within the BSC must be performed to determine the need and method for decontamination.

(d) The most common decontamination method uses formaldehyde gas, although more recently, hydrogen peroxide vapor and chlorine dioxide gas have been used successfully.

(e) In most instances where BSC decontamination is necessary, either depolymerized paraformaldehyde or chlorine dioxide gas should be used. Prior to decontamination with an alternative method (such as VPHP), cycle parameters and validation of those parameters must be developed. When considering decontamination methods, many factors must be taken into account, such as safety, cycle time, effectiveness, equipment costs, and NSF requirements.

(f) Lab personnel should contact Environmental Health and Safety for guidance when determining the need for gas decontamination.

(4) Certification of Biological Safety Cabinets Used for Work Not Requiring Personnel Protection

As previously described, a Class II biological safety cabinet provides personnel, product and environmental protection. Occasionally a laboratory will use a Class II BSC for work that does not require personnel protection (e.g., using BSL-1 agents, PCR work). In these unique situations, a request is sometimes made for a BSC used for this purpose to be exempt from the annual certification. There are multiple justifications for requirements to annually certify all Class II BSCs using a qualified vendor, even when a BSC is used for experiments that do not require personnel protection.

(a) According to the CDC/NIH: “A BSC must be routinely inspected and tested by training personnel, following strict protocols, to verify that it is working properly. This process is referred to as certification of the cabinet and should be performed annually.”

(b) A Class II BSC should be selected for protection of the worker, product and environment. The certification performance testing verifies that a Class II BSC provides all three types of protection. Therefore if a Class II BSC does not pass certification, it will not reliably provide all three types of protection and would not be beneficial for safety or research purposes. The Class I BSC provides personnel and environmental protection, but no product protection. Work that does not require personnel protection may not need to be conducted in a BSC. For instance, PCR can be safely performed in a PCR enclosure or cabinet that protects the sample from contamination. A PCR cabinet is not considered safety equipment, and therefore the EH&S/IBC does not require annual certification (even if recommended by the manufacturer).

(c) EH&S is responsible for promoting the proper maintenance of safety equipment (e.g., BSCs, chemical fume hoods, eyewashes, safety showers) in labs to ensure this safety equipment functions according to its design specifications. EH&S is available to provide guidance on the selection, installation and use of appropriate safety equipment based on a risk assessment. Once a BSC has been installed, it must be certified annually based on its Class and Type.

(d) In the event that a BSC does not pass annual certification, then a sign must be prominently displayed on the front of the sash that indicates – "This Biosafety Cabinet Did Not Pass Certification – DO NOT USE!" When the maintenance or repairs have been completed and the BSC passes certification, the sign can be removed from the sash and the BSC can be used. This procedure is consistent with numerous other academic research institutions that require annual certification of all BSCs to ensure proper operation, regardless of the intended use.

7. PROGRAM ADMINISTRATION

(1) Coordination of certification. Primary responsibility for the coordination of annual BSC certifications is held by the following:

(a) USC-Columbia – USC Environmental Health and Safety

(b) USC School of Medicine (SOM) – USC School of Medicine Health and Safety Services division and SOM Research Safety Coordinator

(2) Funding:

(a) USC Environmental Health and Safety provides funding for annual certification of BSCs in research laboratories at the USC-Columbia campus. The Principal Investigator that owns the BSC must provide funds for expenses associated with initial certification or any relocations or necessary BSC maintenance or repairs (including recertification outside of annual certification). If a BSC does not pass the annual certification, then the sash must be closed and a sign must be prominently posted to ensure the BSC is not used until it has been properly certified.

(b) The USC School of Medicine, Health and Safety Services division provides funding for annual certification of BSCs in research laboratories at the USC School of Medicine. The SOM Health and Safety Services division may charge back individual Principal Investigators or departments for expenses associated with the annual certification and/or necessary maintenance or repairs.

(3) BSC Inventory:

(a) USC Environmental Health and Safety maintains an inventory of BSCs for USC-Columbia and the USC School of Medicine. Each BSC's class and type, certification date, serial number, and location are included in the database. Certification dates are annually reported to EH&S so the database can be updated.

(b) All new BSC installations should be reported to EH&S to include in the inventory records.

(c) EH&S will share this inventory with the designated certification vendor to ensure all BSCs are certified annually and certification records are accurately maintained.

8. RESPONSIBILITIES

(1) School of Medicine, Health and Safety Services division and SOM Research Safety Coordinator

a) Coordinates annual certification for BSCs at the USC School of Medicine (SOM).

b) Ensures BSC certification vendor receives timely payment for services provided in SOM labs.

c) Reports to EH&S any BSC maintenance/repairs, gas decontamination post-repair certifications, and BSC relocations or new installations.

(2) Environmental Health and Safety

a) Maintains an updated inventory record of all BSCs in laboratories at each USC campus.

b) Coordinates with the certification vendor to ensure all BSCs are certified annually.

(3) Principal Investigators/BSC Users

a) Ensure the implementation of appropriate work practices when using a BSC, consistent with this program and the USC biological safety training.

b) Promptly report any problems with the selection, installation, or use of BSCs to the USC Biological Safety Officer (BSO). The BSO can provide guidance on these issues or can assist with coordinating maintenance or repairs. The PI must ensure their BSCs pass certification. If a BSC does not pass certification, the PI must ensure it is not used until it is certified.

c) Report the relocation or new installation of BSCs to the USC Biological Safety Officer. Any BSC that has been relocated or is a new installation must be certified prior to use. Proper reporting will also ensure the BSC certification inventory accurately reflects the location of each cabinet requiring annual certification.