



Biological Safety Cabinet Operations

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Objective

To describe policies and procedures related to daily use, cleaning, decontamination, routine maintenance, and annual certification of Biological Safety Cabinets (BSCs).

Definitions and Acronyms

Aerosol-producing Activities: May include, without limitation, opening containers with non-ambient pressures; intranasal inoculation of animals; harvesting infected tissues/fluids, or embryonate eggs; transfer operations; necropsy of infected animals; changing animal cage bedding and operating aerosol-producing equipment.

Aerosol-producing Equipment: May include, without limitation, vortexers, blenders, sonicators, centrifuges, grinders, vigorous shakers, and mixers. However, equipment (e.g. sealed rotors, buckets or centrifuge safety cups) that is designed to contain aerosols does not have to be operated in a BSC, provided that the containers (e.g. rotors or centrifuge safety cups) are opened only in a BSC.

Biological Safety Cabinet (BSC): Primary containment device which utilizes HEPA filtered directional airflows to contain potentially infectious materials during experimental procedures. The BSC provides protection for the surrounding environment, research personnel and research materials being manipulated.

Laminar Flow Benches (LFBs): Also referred to as clean benches. Provide an aseptic environment for experimental work by passing HEPA filtered air across the work surface. The Department of Biological Safety actively discourages the purchase and use of LFBs since air is blown across the work surface into the face and torso of the operator. The Institutional Biosafety Committee and the Department of Biological Safety recognize that clean benches do not provide personnel or environmental protection from infectious or potentially infectious agents, allergens, chemicals or radioactive materials. If you are using a clean bench, contact the Department of Biological Safety for a review of your procedures.

National Sanitation Foundation (NSF): Regulatory agency responsible for development of standards associated with the certification and testing of BSCs.

Primary Containment: The engineering control which prevents the release of potentially infectious material into the laboratory or outside environment. The use of a primary containment device, such as a BSC, prevents contamination of the room.

Background Information

Engineering controls, such as biological safety cabinets (BSCs), reduce the risk of employee exposure by removing or isolating the worker from the hazard. Biological safety cabinets (BSC)

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are the primary means of containment for personnel working with infectious agents and other biohazards. BSCs are only one part of an overall biosafety program that requires consistent use of good microbiological practices. The efficacy of BSCs depends upon the behavior of the operator, the unit's orientation in the facility, and the movement of personnel in the laboratory. Personnel must use appropriate practices and procedures while working in a BSC for the cabinet to contain potentially infectious splashes and aerosols, which are generated by many experimental procedures. Personnel must be adequately trained in the use of biological safety cabinets prior to use.

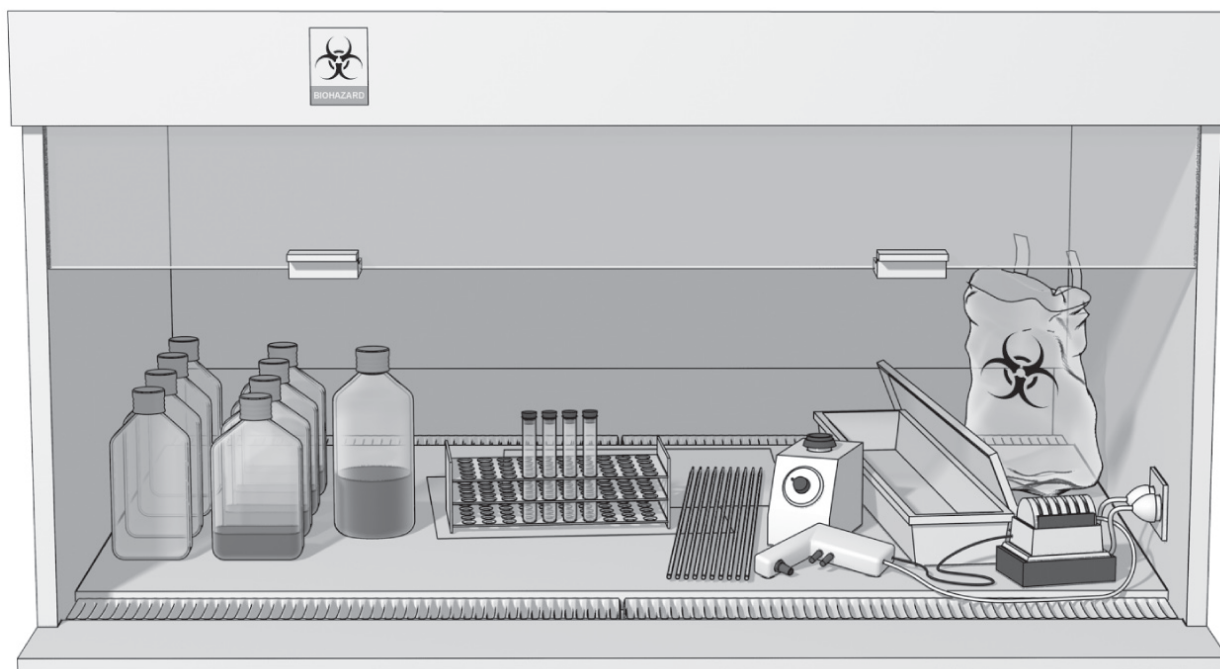
BSC exhaust air is passed through a certified high efficiency particulate air (HEPA) filter, which is effective at trapping particulates and infectious agents. The exhaust air from the BSC is either re-circulated back into the laboratory or exhausted out of the building. BSCs that re-circulate air into the room shall not be used for work with volatile or toxic chemicals as health and safety hazards can result from the buildup of chemical vapors in the cabinet and laboratory.

Procedure

Use of Biological Safety Cabinets (BSC)

- Conduct all procedures involving the manipulation of potentially infectious materials, including aerosol-producing activities, when using aerosol-producing equipment, or when using high concentrations/large volumes of organisms, inside a biological safety cabinet.
- Storage of excessive materials or equipment inside a BSC can disrupt airflow, resulting in turbulence, cross-contamination, or breach of containment. Therefore, only materials and equipment necessary for immediate work will be placed in the BSC. Additionally, storing items on top of the BSC can interrupt exhaust airflow and can result in damage to the BSC. Place large items close to the sidewalls, rather than at the back of the cabinet where they will interfere with airflow. Blocking the intake grills in the front and rear of the cabinet will interfere with proper functioning of the cabinet, which can cause a loss of containment of infectious organisms.
- Prior to each use examine the cabinet to ensure it is clean and in good repair. Verify that the cabinet blower is on and functioning properly based upon observance of Magnehelic gauges and/or digital readouts. Ensure that UV lights are disengaged prior to commencing work in the BSC.
- Wipe interior of BSC (work surface, walls & interior surface of window) and items placed in the cabinet with an efficacious and approved disinfectant.
- Ensure the cabinet drain valve is closed prior to starting work, to contain spills.

Place items necessary for the procedure (including a receptacle for waste) in BSC prior to initiating work. This will minimize the number of arm-movement disruptions to the air barrier, which can compromise the containment of the BSC. Arrange the materials within the cabinet in a manner to ensure good aseptic technique; e.g., all workflows from one side of the cabinet (clean) to the other side (dirty). Contaminated items never cross over uncontaminated/clean supplies. Below is a figure from *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets*, 3rd Edition which demonstrates the concept of loading the cabinet and working from clean to dirty.



- Verify that the sash is set at the appropriate height. The operating height of a BSC is not variable; so follow the manufacturer's recommendations for the BSC that you are using.
- Cabinets are designed for a single operator. More than one person working in the BSC at one time (even in a six-foot BSC) produces enough disturbances in the airflow to breach the containment of the BSC. Never lean into a BSC or place head into a BSC.
- The BSC must be emptied and decontaminated at the end of procedure and/or workday. Every individual is responsible for cleaning the BSCs when they have finished working. This cleanup involves the following steps:
 - Remove all items from the biosafety cabinet. Surface decontaminate the exterior of all potentially contaminated material prior to removing from cabinet.
 - Place all waste in an autoclave bag in the biological safety cabinet and seal prior to removing.
 - Wipe the interior surface of the cabinet with an approved disinfectant after all items have been removed from the cabinet.
 - Do not rely on UV light as a means of decontamination. A complete discussion of the shortcomings of UV light as a decontaminant can be found on the UK biosafety website.
- Avoid common mistakes associated with use of biological safety cabinets:
 - Air currents and drafts can disrupt BSC airflows. Locate BSCs away from doors, vents/diffusers and traffic paths.
 - Do not store equipment or supplies inside the cabinet.
 - Make sure that items necessary for procedures are inside the BSC prior to starting work in the BSC.
 - To prevent disruption of airflow and damage to the HEPA filter, do not store items on the top of the cabinet.
 - Prevent damage to cabinet by keeping all objects (i.e. paper towels, Kim Wipes, work surface diapers, etc.) from being pulled into the back, front, and side grills or slots.

- Never disengage the alarm. The alarm indicates when the cabinet has improper airflow or reduced performance, which may endanger the researcher and/or the experiment.
- Avoid rapid motions at the front of the unit and minimize movement of arms in and out of cabinet which may disrupt the air curtain.
- Protect vacuum lines by using an appropriate trap system with primary and overflow flasks containing an approved disinfectant and a hydrophobic HEPA filter between the trap system and the vacuum pump or house vacuum system. Below is a figure from *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets*, 3rd Edition which provides an example of a proper aspiration system showing the catch flask (A) and overflow flask (B) prefilled with disinfectant and the inline filter (C) protecting the vacuum source (D). The entire system should be contained in an autoclavable pan or similarly vessel to prevent spills in case of a broken flask.



Routine Maintenance of Biological Safety Cabinets (BSCs)

- Routine maintenance of BSCs will be performed as recommended by the cabinet manufacturer.
- All maintenance and repairs will be performed by BSC certification and maintenance contractors.
- The PI is responsible for any service or repairs that are needed.
- A risk assessment will be completed prior to maintenance or repairs to determine the extent of decontamination required prior to commencement of work. The Biological Safety Officer will determine the type of decontamination necessary. Typically surface decontamination is adequate, however, there are instances such as extensive repair inside the cabinet envelope or HEPA filter replacement that will require gas decontamination.
- Recertification of the BSC may be required based upon the type of maintenance or repair work that was completed. The Biosafety Officer will determine the need for recertification.
- Copies of maintenance or repair reports will be maintained by the Principal Investigator or Laboratory Manager.

Annual Certification of Biological Safety Cabinets (BSCs)

- Biosafety cabinets will be certified at least annually by NSF-certified technicians and according to NSF 49 standards.
- All BSCs and LFBs at the University of Kentucky shall be placed in the University of Kentucky Department of Biological Safety inventory database.
- Any BSCs or LFBs not certified will be reported to the Department Chair, Executive Vice-President of Research, and Provost as a serious lab violation.
- It is the responsibility of the Principal Investigator (PI) to participate in the required annual certification process.
- Prior to certification the user will be responsible for decontaminating the interior surfaces of the cabinet using an approved method and disinfectant specific to the agent.
- Copies of annual certification reports will be maintained by the Principal Investigator or Laboratory Manager.

Movement, Storage, Surplus or Disposal of BSCs

- Notify the Biosafety Office in advance when you plan to have BSCs or LFBs moved, placed in storage, transferred to a new owner, discarded, removed from the University of Kentucky, or obtained from another institution or manufacturer.
- The PI is responsible for ensuring proper decontamination of the BSC or LFB.
- BSCs shall be professionally gas or vapor decontaminated by a certified technician, before a unit is relocated, stored, serviced (interior), or discarded based upon the agents which have been manipulated in the cabinet and the future usage of the BSC. Contact the Department of Biological Safety for a risk assessment based upon the use/reuse of the cabinet to determine the appropriate decontamination method.
- BSCs must be recertified after movement prior to use.

Summary of UK Biological Safety Cabinet Usage Guidelines

- User should receive training prior to using BSC
- Check to see BSC has been certified annually
- Do not use BSC if any alarms are activated
- Only one person should work in BSC at a time
- Use of hazardous chemicals, radioactive isotopes or open flame STRICTLY prohibited in BSC without prior approval from Biological Safety Department
- Do not use BSC as a storage area
 - Overloading BSC with unnecessary items can affect airflow and containment
- Purge BSC before and after work
 - Turn blower on 15 minutes before work begins and allow to remain on 15 minutes after work has concluded
- Wear proper PPE when using BSC
 - At minimum lab coat and gloves should be worn during work
- Perform complete surface decontamination
 - Decontaminate BSC interior before and after each use with disinfectant approved for agent(s) in use
- Always open sash to correct working height
- Observe proper aseptic and microbiological technique
 - Plan work before starting and place all items inside the BSC before beginning. Work flow should proceed from clean to contaminated area

- Work within safety area
 - Do not obstruct any air grilles in front or back of work zone. Work as deep in the work zone as possible
- Know the effects of movement
 - Work in a controlled and steady manner. Move arms straight in and out of BSC. Rapid movements can compromise containment. Air flow from doorways, human traffic and room air ducts can all compromise containment
- Disinfect all materials removed from BSC
 - Contaminated waste generated during work must be decontaminated before disposal

References

NSF/ANSI Standard 49 Class II (laminar flow) biosafety cabinetry
http://www.nsf.org/newsroom_pdf/bc_biosafety_cabinetry_testing.pdf

Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, 3rd Edition
https://www.cdc.gov/biosafety/publications/bmbl5/bmbl5_appendixa.pdf

Baker Company, Introduction to Biological Safety Cabinets
<http://bakerco.com/intro-to-biological-safety-cabinets.html>