Biological Safety Cabinet (BSC) SOP

Background

This document establishes general standard operating procedures for using a Biological Safety Cabinet (BSC) in the laboratory for biohazard containment.

Biological Safety Cabinets (BSCs) are enclosures that control biohazardous aerosols in labs. The Occupational Safety and Health Administration (OSHA), Centers for Disease Control and Prevention (CDC), the National Institutes of Health (NIH), and the National Sanitation Foundation (NSF) set requirements for BSCs used to protect lab personnel and the environment. BSC's are required for use in laboratories working with Biosafety Level 2 or above material.

Definitions

Biological Safety Cabinet (BSC): A piece of equipment with controlled airflow and HEPA filters so that it can perform two functions:

a. Provide a sterile work environment so that the work is not contaminated by adventitious agents

b. Contain biohazardous aerosols to protect lab personnel and the environment.

Engineering Control: A term used by the Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH); engineering controls protect workers by removing hazardous conditions or by placing a barrier between the worker and the hazard. In OSHA's hierarchy of controls, engineering controls should be used before Administrative (work practice) Controls and Personal Protective Equipment.

HEPA filter: High Efficiency Particulate Air Filter, a fiber filter that captures at least 99.97% of a 0.3 micron particle (greater capture efficiency for smaller or larger particles) and provides laminar airflow (airflow moving in one direction without turbulence). HEPA filters, however, do not capture chemical vapors or gases.

Primary Containment: A term used by the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH); primary containment is any piece of equipment that protects lab personnel from biohazardous aerosols, splashes or sprays.

Responsibilities

It is the responsibility of the Principal Investigator and/or the Laboratory Supervisor to ensure that his/her staff and students adhere to these requirements as a minimum and that the Biological Safety Cabinet (BSC) is certified through the University service representative as required.

It is the responsibility of each person operating the Biological Safety Cabinet (BSC) to adhere to these

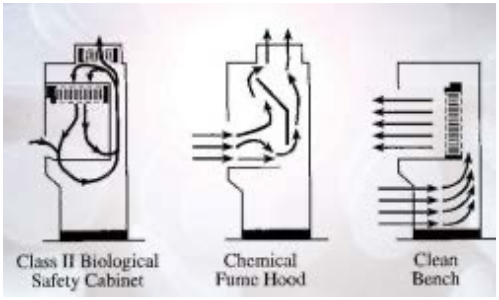
requirements and make their supervisor aware of any issues they encounter while using the BSC.

Procedures

I: Use and Limitations

BSCs serve as Engineering Controls in OSHA's hierarchy of controls, and as Primary Containment for CDC/NIH.

BSCs are commonly referred to as 'hoods' in the lab, but 'hood' is a general term that refers to BSCs, chemical fume hoods, clean benches, PCR hoods, vertical laminar flow hoods, etc. While a 'hood' is any enclosed work surface with controlled airflow that personnel can place their arms in and perform experiments, each type is designed differently based on the use or hazards they control. Therefore, lab personnel must know what hazards they work with and what 'hood' to use or not use.

'Hood' Type	Use/Limitations	Airflow Schematic
Biological Safety Cabinet (BSC)	Used for biohazards. Few (if any) chemicals	HEPA Filters No HEPA HEPA Filter 
Chemical Fume Hood	Used for Chemicals Not biohazards.	
Clean Bench, PCR Hood, Laminar Flow Hood	Used for sterile work. Not for hazards.	

Picture reference:
Adapted from American Chemical Society's Division of Chemical Health and Safety, Fume Hood Usability Considerations
https://acsdchas.wordpress.com/workshop-report-summary/4-operational-parameters/

Visual demonstrations of 'hood' airflows:

- Airflow Patterns in Biological Safety Cabinets and Laminar Flow Hoods by the Viral and Human Genomics Laboratory, Universidad Autonoma de San Luis Potosi
  - The first 2 minutes utilize CO_2 vapor to demonstrate airflow.
- Certified Biological Safety Cabinet Animation by Thermo Scientific EN 12469
  - 3 minutes showing in the inner workings of the biosafety cabinet.
- Mythbuster Series of videos by the Baker BSC Company
  - Common laboratory myths and thoughts about BSC usage explored.

II: Certification, Gaseous Decontamination, and Repairs

Biological safety cabinets (BSCs) are field tested in accordance with NSF/ANSI 49 and manufacturer's specifications by a university approved vendor, Technical Safety Services (TSS). The certification process verifies the inflow and downflow directions and velocities as well as testing the HEPA filter integrity.

- To request BSC certification, internal BSC repair, or decommissioning at Rutgers utilize the "BioSafety Cabinet Service Request" link on the REHS Homepage under the "Helpful Links" section in the left column.

CDC/NIH and OSHA require BSCs to be certified:

- When first installed
- Every 12 (twelve) months
- After repairs
- When moved - due to potential HEPA filter shifting or damage occurring
  - Moving a BSC should only be done with Rutgers Facilities assistance.

For repairs:

- The certifier (TSS) performs all repairs inside the BSC - such as the motor(s), HEPA filter(s), lightbulbs, or any potentially contaminated surfaces inside the cabinet etc.
- Rutgers Facilities performs any repairs outside the BSC – such as building ventilation, electrical supply etc. Place a Facilities Work Order to have these types of repairs accomplished.

To dispose of or decommission a BSC:

- The parts of the BSC that lab personnel can reach, should be decontaminated following normal laboratory procedures (10% bleach or other suitable reagent)
- The contaminated parts of the cabinet (including the HEPA filter) that lab personnel can't reach must be decontaminated using gaseous paraformaldehyde or vaporized hydrogen peroxide. The cabinet certifier (TSS) performs this required decontamination.

III: Work Practices

General Principals of working in a BSC:

- The proper use of a Biological Safety Cabinet (BSC) complements good microbiological techniques which together will result in containment and control of infectious agents. As with all lab equipment, personnel must be trained in the proper use. General working instructions for the safe and effective daily use of BSCs are outlined below.
- Read the manufacturer's Operator's Manual to know the specific features and alarms unique to the BSC. The BSC manufacturer and model number will be located on the BSC.
- Do not use chemicals in a biological safety cabinet (BSC), it is not the same as a chemical fume hood. Most BSC types blow the HEPA-filtered air back into the room and will not trap chemicals in vapor form. Small amounts of chemicals common to biological research (e.g. TRIzol) may be used, however, ensure they are used in small quantities and keep chemical stock bottles closed when not in use. If unsure about usage of a volatile or toxic chemical or biological toxin, contact REHS for a risk determination before using a in a BSC.
- Do not use flammable materials in a BSC if they can be avoided. Most BSC types recirculate the air within the cabinet. This recirculation can concentrate chemicals, and flammable chemicals like ethanol can get into the explosive concentration range (Lower Explosive Limit). Click here for a picture of a BSC fire.

- Do not use open flames. Even if there is no explosion risk, flames may damage the HEPA filters. Use sterile supplies and good, sterile technique to prevent contamination. Click here for 4 reasons not to use flames in a BSC. If needed, ask REHS about electric alternatives to Bunsen burners.
- In 4 feet BSC's, due to airflow requirements, only one person may work at a time. It is not recommended practice but if it is necessary, a 6 foot Class A2 cabinet with an 8" sash height (cannot be 12") may accommodate two people working with the same organism.

General Working Instructions for daily use of a BSC:

1. Prepare for work in the BSC by planning and gathering all materials and supplies that will be required for that day's tasks. Create a checklist if needed to reduce the number of times the protective air current will be broken by entering the cabinet. This will reduce the contamination risk and increase the effectiveness of the BSC in keeping containment.
2. Ensure the BSC is operational and ready for work.
    a. Check that the certification sticker date is within the last year. If it is not, notify your supervisor.
    b. If there is a UV light present, confirm it is off.
    c. Confirm that the BSC alarms are on and functioning appropriately.
    d. Turn on working lights, electricity, and the cabinet blower. Allow the BSC blower to operate for at least 5 minutes prior to starting work to purge any contaminants from the air in the cabinet.
    e. Check the magnehelic gauge (or electronic display) for the differential pressure (Δp or dp) reading. Compare with the reading on the certification sticker; it should be +/- 10%. Low readings could possibly mean the protective air curtain is compromised while high readings could potentially indicate the HEPA filter is loading compromising airflow. Notify supervisor if either is observed as the BSC may not be providing efficient containment.
    f. If applicable for liquid waste, check aspirator system to ensure that the primary collection flask is less than ½ full and contains sufficient 10% bleach in the bottom. An inline hydrophobic 0.22 micron filter is required to protect the vacuum system. Flasks must be placed in a secondary container if stored outside the BSC. Secondary spill-over flaks are highly recommended but not required at Rutgers.
3. Don personal protective equipment (PPE) sufficient for the material(s) being worked with.
    a. Lab coats button closed, or solid front gowns should be worn over street clothing while working in the BSC.
    b. Gloves should be worn and if possible, the usage of long-cuffed gloves for extra protection should be pulled up over the cuffs of the lab coat. Double gloves maybe worn if additional layers are required, or frequent glove changes are anticipated.
    c. Lab safety glasses or googles should still be worn even with the proper usage of the BSC safety sash in place at the correct working height.
    d. Plastic or Tyvek disposable lab sleeves maybe considered as an additional layer of protection based on material(s) being used and sterility requirements.
4. Adjust chair so armpits are at the same level as the bottom of the sash.
5. Disinfect the BSC working area by wiping the work surface, interior walls, and the inner surface of the sash with a suitable disinfectant, such as 70% ethanol or an idophor based solution.

a. Bleach is highly corrosive and should be avoided unless cleaning a spill and then cleaning must be followed with distilled water to remove excess bleach. Use of bleach without adequate removal, will cause pitting and rust the stainless steel.
b. Do not saturate the grilles heavily with disinfectant as the mist can be drawn into the HEPAs compromising them.
c. The work surface can be removed to conduct a deep cleaning of the BSC. Lint, debris, sharps, and micropipette tips can all build up in the catch basin beneath the work surface. This area should be cleaned periodically. When removing the work surface, wear thick gloves to protect hands from sharp edges.
    1. Removing the BSC Working Surface - Arizona State University
        a. Start at 14:10
    2. Deep Cleaning a BSC2

6. Setup the BSC for the work and experiments being conducted that day. Limit what is brought into the BSC to what is needed for completing work. Do not use the BSC for storage to avoid unnecessary, additional, airflow impediment.
    a. All entries into the BSC should be done perpendicular to the front grille to minimize airflow disturbances.
    b. Place equipment and supplies with relation to the front and back grilles. Do not block airflow to any BSC grilles.
        i. Front: Everything should be placed at least 4 inches back from the front grill area. Nothing should be placed on the front grill area, including pipettors, notes, elbows etc.
        ii. Back: Any equipment that has the potential to generate aerosols (microcentrifuge, vortex, blender etc.) should be kept near the back 1/3rd of the cabinet. Nothing should be pushed against the back of the hood, including pipette tip boxes, petri dish bags, vortexers, etc.
    c. Arrange supplies in a logical order; working from clean (left) to dirty (right) and utilizing the three-zone flow of work. If left-handed, considering working in the opposite direction.
        i. The clean area contains media, sterile supplies, etc.
        ii. The working area is in the center of the BSC on the working surface.
        iii. The contaminated area contains waste collection trays/beakers.

Picture reference:
Adapted from the Canadian Biosafety Handbook, Second Edition | Chapter 11-15
https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/handbook-second-edition/chapter-11-15.html

    d. Plastic-backed absorbent pads or a disinfectant-soaked towel may be placed in the working area to contain any spills or splatters that may occur but ensure that it does not block any airflow grilles.
    e. Wipe each item with proper disinfectant prior to placing in BSC to reduce introduction of contaminants.
    f. Prepare the waste discard area with appropriate collection method(s) for waste being generated, for instance a beaker containing 10% Bleach for serological pipets.
7. While working, limit the disruptions to the protective air current.
    a. Introduce arms/hands perpendicular to the front grille/air curtain. Do not swing arms in and out of the BSC while working.
        i. Place all supplies (ie pipet tips etc.) inside the BSC on the clean side while working
        ii. Utilize the waste discard area inside the BSC on the contaminated side.
    b. While placing arms/hands inside the BSC, allow a few moments for the airflow to stabilize before continuing work.
    c. Keep the laboratory door closed and be aware of people walking within 6ft of the BSC sash as these can disrupt the airflow.
    d. For monitoring air flow disruptions – tape a one inch strip of Kimwipe to the sash edge.
8. Avoid touching the front edge of the cabinet with contaminated gloves. If pushing away or scooting closer to the BSC is necessary, use feet or remove gloves before touching the front edge of the BSC. This surface is in contact with the front of the lab coat and is a potential area of contamination.
9. Once work is completed dispose of accumulated waste from inside the BSC by following proper RMW procedures, wipe down all items with an appropriate disinfectant and remove from BSC. Do not utilize the BSC as a storage area. Disinfect the working surface, the sides and back of the cabinet, and the inner and outer parts of the sash; thoroughly cleaning around and under everything. Check the fullness of the aspirator system, following proper disposal methods to empty if more than ¾ full.
10. Follow manufacturing and building recommendations on keeping the BSC blower on or turning if off once work is completed. If unsure, check with REHS. If turning off blower, allow the BSC to operate after disinfecting the cabinet for five minutes with no activity inside the cabinet to purge any residual biohazardous aerosols.
11. Prior to leaving the lab, doff all PPE disposing of contaminated items in the appropriate fashion, and thoroughly washing hands. Do not wear gloves or lab coats outside of the lab or into common use non-laboratory areas.

Visual Demonstrations of Working in a BSC:

- Fundamentals of Working in a Biological Safety Cabinet – CDC Online Course
    - 1 hour; there's also a Virtual Reality version
    - CDC TRAIN is another great CDC resource that requires users to setup a free account to complete online lab training courses.
- NIH National Biosafety and Biocontainment Training Program -Biological Safety Cabinet (BSC): How it Works to Protect You
    - 5 minutes
- Effective Use of Class II Biological Safety Cabinets (Digital Training Package) Eagleson Institute

        - 3 minute preview
- Working Safely BSC: Proper Preparation, Steps to Take Before You Begin Work (Nuaire)
        - 5 minutes
- Work from Clean to Contaminated (Nuaire)
        - 3 minutes

VI. References

- Baker Company Mythbusters https://bakerco.com/communication/bsc-mythbusters/
- Canadian Biosafety Handbook, Second Edition | Chapter 11-15; https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/handbook-second-edition/chapter-11-15.html
- CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 6th edition, 2020, Appendix A, Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets https://www.cdc.gov/labs/BMBL.html
- Eagleson Institute, Safe Use of Biological Safety Cabinets or The Case of the Contaminated Cultures https://www.youtube.com/watch?v=IN9_0V0gOBo (22 minutes)
- Labconco article – 4 Reasons NOT to Use Open Flames in Biosafety Cabinets https://www.labconco.com/articles/4-reasons-not-to-use-flames-in-bscs
- Meechan, P., Wilson, C. Use of Ultraviolet Lights in Biological Safety Cabinets: A Contrarian View. Applied Biosafety 2006, 11 (4): 222-227 http://journals.sagepub.com/doi/abs/10.1177/153567600601100412
- NIOSH Directory of Engineering Controls www.cdc.gov/niosh/engcontrols/default.html
- NSF/ANSI Standard 49 - Biosafety Cabinetry: Design, Construction, Performance, and Field Certification, 2019, Annex I-1 https://d2evkimvhatqav.cloudfront.net/documents/nsf_49__annex_I-1.pdf
- Princeton, Biological Safety Cabinets (includes Baker one page BSC guide) https://ehs.princeton.edu/laboratory-research/biological-safety/biological-safety-cabinets
- Stanford Biosafety Manual https://ehs.stanford.edu/manual/biosafety-manual/biological- safety-biosafety-cabinets
- University of Rochester Design Standards Section 15870 – Biological Safety Cabinet Standard (2022)

Create a safe working environment in your Biological Safety Cabinet

1. AIRFLOW PROBLEM

The Magnehelic gauge should read close to the setting at the last certification (record that reading for easy reference). A reading that's too high or low may indicate a problem with the airflows.

- Don't work in the cabinet if the Magnehelic gauge indicates a problem. Call for service.

2. MIXING CLEAN WITH DIRTY ITEMS

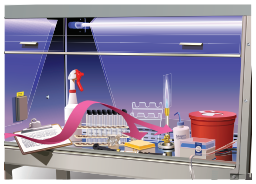
Take care to separate clean items from dirty inside the cabinet. Use a systematic process (i.e. left to right or front to back) to avoid contamination.

- Limit items inside the cabinet to only the most essential.

3. COVERED AIR INTAKE GRILLE

When untreated laboratory air is blocked from entering the front grille, it can flow over the work surface contaminating the product and posing a risk to personnel.

- Never block the front air intake grille.



4. CLIPBOARD IN CABINET

Anything placed into the cabinet becomes contaminated—pens, pencils, clipboards, etc. If this occurs, always disinfect the item before taking it out.

- Use only the items essential to your task in the safety cabinet.

5. IMPROPER PIPETTE AND WASTE DISPOSAL

Pipettes should be decontaminated within the cabinet. Disinfect items in a shallow pan filled with disinfectant. Other biohazard waste should be similarly bagged in the cabinet to avoid spreading contamination.

- Remove disinfected waste from the cabinet frequently.

6. EXHAUST DIFFUSER MISSING OR NOT INSTALLED

Any Class II Type A cabinet not connected to an exhaust system uses an air diffuser that also protects the exhaust HEPA filter. This filter is very fragile and easily damaged. Make sure the diffuser is installed correctly and not blocked. Keep the top of the cabinet clear.

- Refer to your operator's manual for details.

7. SASH ALARM MUTED

An alarm sounds and flashes when the sash is opened too high or closed too low. The alarm can be silenced seven minutes at a time for loading large equipment and cleaning.

- Never work in the cabinet when the cabinet is in alarm.

8. UV LIGHT WHEN SASH IS OPEN

UV radiation is hazardous to your health. Newer cabinets have a safety circuit that allows the UV light to activate only if the sash is completely closed. Do not adjust this important setting.

- Make sure the sash is completely closed before using the UV light. The glass sash will protect you from UV radiation.

9. BUNSEN BURNER (OPEN FLAME)

Flammable gasses (gasses are NOT captured by HEPA filters) may recirculate and build up to the Lower Explosive Level (LEL) and cause an explosion or fire.

- Find a substitute. The heat from an open flame can disrupt airflows.

AIRFLOW SCHEMATIC FOR CLASS II TYPE A2 BIOSAFETY CABINETS

- Room air
- HEPA filtered air
- Contaminated air - negative pressure
- Contaminated air - positive pressure