UNIVERSITY OF ROCHESTER
ENVIRONMENTAL HEALTH & SAFETY

Policy No.: BS010	**Approved by: UR IBC**
Title: Requirements for Biological Safety Cabinets Used in Laboratories for Biohazard Containment	**Date: January 12, 2022**
Revision No.: 3	**Page 1 of 11**
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I. PURPOSE

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.

This procedure establishes the minimum requirements for maintaining and operating BSCs used in labs working at Biosafety Level 2 or above.

II. PERSONNEL AFFECTED

University of Rochester research and clinical lab personnel who operate Biological Safety Cabinets (BSCs) and their supervisors

Environmental Health and Safety staff who audit labs

III. DEFINITIONS

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

1. Provide a sterile work environment so that the work is not contaminated by adventitious agents, and
2. 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.

UR IBC: University of Rochester Institutional Biosafety Committee, composed of faculty, staff, and community members; required by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

IV. RESPONSIBILITIES

It is the responsibility of the Principal Investigator and the Laboratory Supervisor to ensure that his/her staff and students adhere to these requirements as a minimum.

It is the responsibility of each person operating Biological Safety Cabinets (BSCs) to adhere to these requirements.

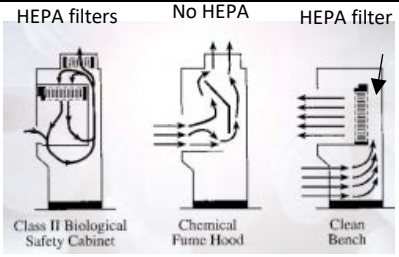
It is the responsibility of Environmental Health and Safety to periodically audit labs working at Biosafety Level 2 and above to ensure that labs adhere to these requirements.

V. PROCEDURES

A. Use and Limitations

As mentioned in Purpose, 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 more 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 to 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	Used for biohazards Few (if any) chemicals (more allowed in B2s)	
Chemical Fume Hood	Used for Chemicals No biohazards (case-by-case approval)	
Clean Bench, PCR Hood, Laminar Flow Hood	Used for sterile work No hazards	

Note: Radioisotopes are not included in the above table; use is evaluated by EH&S's Radiation Safety Unit.

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:

- Viral and Human Genomics Laboratory, Universidad Autonoma de San Luis Potosi, Airflow Patterns in Biological Safety Cabinets and Laminar Flow Hoods https://www.youtube.com/watch?v=Wg61LdngWlQ (the first 2 minutes follow CO2 vapor in different places)

ThermoScientific EN 12469 Certified Biological Safety Cabinet Animation https://www.youtube.com/watch?v=oIuWQqzw324 (3 minutes-have you ever wanted to see behind the work surfaces and how it all works?)

B. Selection, Placement, and Installation

When selecting a BSC, EH&S in conjunction with the PI selects the appropriate BSC type. Several Classes and Types are available with different airflows and HEPA placement. The most common type used for biohazards is the Class II Type A2 (aka A/B3). The older A1 has a positive plenum, which must be part of the risk assessment.

Biological Safety Cabinet Class/Type	Connected to building exhaust ventilation?	Allowable usage of flammable or volatile toxic chemicals (determined by UR's Chemical Safety Officer)*
Class II, Type A2 (aka A/B3)		None*
Class II, Type A2 (aka A/B3)	Yes, using thimble connection (has air gap)	Minute quantities
Class II, Type B2 (works like a chemical fume hood)	Yes, using hard connection (similar to a chemical fume hood)	- Small quantities - No chemicals that may compromise the HEPA filter or its seal

* HEPA filters capture particulates/aerosols, but do not trap chemical vapors/gases. Therefore, any chemical vapors are exhausted into the lab unless the BSC is connected to the building's exhaust ventilation system (you would see a duct 'attached' to the exhaust HEPA at the top of the cabinet, and for thimble connections, there is an additional alarm to detect if the building exhaust is not functioning normally.)

For energy efficiency, Class II Type A2 BSCs recirculate the air blown onto the work surface. Any volatile chemicals are continuously recirculated and therefore, concentrated in the work surface. If flammable chemicals are used, the Lower Explosive Limit can be reached.

Table reference:
UR's Design Standards Section 15870 – Biological Safety Cabinet Standard (2019)

Additional options include ultraviolet (uv) lights for the work surface and natural gas connections. Neither are recommended by EH&S:

- Ultraviolet (uv) light can burn skin and eyes, damage materials left in the cabinet, and does not get under surfaces. Lab personnel must also keep the bulbs dust-free and make sure the wavelength and output is checked once a year.
- Natural gas hook ups should not be used for Bunsen burners. Flames can damage the HEPA supplying sterile air to the work surface and can cause significant fires (especially if flammables such as ethanol are used on the work surface, due to air recirculation in Class II Type A2 BSCs).

When determining BSC location, consider that opening doors into small rooms, other equipment with its own airflow and persons walking within 6 (six) feet behind someone

working in the cabinet can pull air out of the cabinet (if the movement overcomes the inward directional air curtain under the sash, generated by the front grille). Place BSCs:
- where building supply ventilation will not push against the exhaust coming out of the BSC exhaust, and
- with limited movement behind the person working at the BSC.

Installation is performed by non-lab personnel.

For more on the selection, placement and installation of BSCs, refer to UR's Design Standards Section 15870 – Biological Safety Cabinet Standard (2019)

C. Certification, Gaseous Decontamination, and Repairs (primarily by outside contractor)

Certification is verifying airflow velocities and that the filters still meet HEPA requirements.
- UR's list of certifiers: http://www.safety.rochester.edu/ibc/CabsHoodsResource.html

CDC/NIH and OSHA require BSCs to be certified:
- when first installed,
- every 12 (twelve) months,
- when moved (the HEPAs may shift or become damaged), and
- after repairs.

Before the cabinet is moved and before most repairs, the contaminated parts of the cabinet that lab personnel can't reach must be decontaminated using gaseous paraformaldehyde or vaporized hydrogen peroxide. The cabinet certifier performs this decontamination.

For repairs:
- The certifier performs all repairs inside the BSC, i.e. the motor(s), HEPA filter(s), or any potentially-contaminated surfaces inside the cabinet.
- UR Facilities performs any repairs outside the BSC (e.g. building ventilation, electrical supply).

D. Work Practices:

The proper use of a Biological Safety Cabinet (BSC) complements good microbiological techniques and results in effective containment and control of infectious agents. As with any other piece of lab equipment, personnel must be trained in its proper use. General guidelines for the safe and effective use of BSCs are below.

Read the manufacturer's Operator's Manual so you know the specific features and alarms unique to your BSC. The BSC manufacturer and model number will be on the BSC.

Do not use chemicals in a biological safety cabinet (BSC) as you would in 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

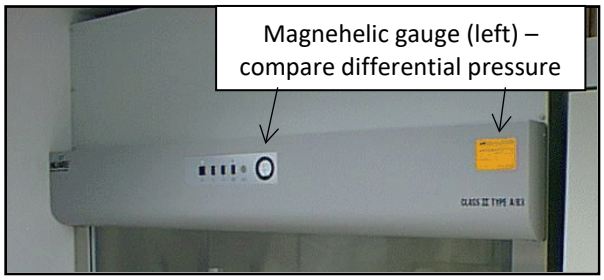
research (e.g. TRIzol) may be used, but use small quantities and keep chemical bottles closed when not in use. Contact the EHS Laboratory Safety Unit for a risk determination before using a volatile or toxic chemical in a BSC.

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). For a picture of a BSC fire, see the bottom of https://ehs.stanford.edu/manual/biosafety-manual/biological-safety-biosafety-cabinets

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. If needed, ask EH&S about electric alternatives to Bunsen burners.
https://www.labconco.com/articles/4-reasons-not-to-use-flames-in-bscs

1. Plan and prepare for your work in the cabinet by having a checklist of materials needed to place in the cabinet before beginning your work. This reduces the number of times you break the protective air current and thereby reduces the amount of contamination entering the cabinet.
2. Only one person should work at a 4 foot cabinet. Two will compromise the airflow.
   - Two people can work with the same organism in a 6 foot Class II A2 cabinet with an 8" sash height (but not a 12"). However, consider what would happen if hands or elbows bumped into each other.

     https://bakerco.com/communication/bsc-mythbusters/ - Can multiple people work safely in a BSC?
3. Check the certification sticker and the magnehelic gauge (or electronic display).
   a. The date should be within the last year.
   b. The Δp or dp (differential pressure) reading on the sticker should be +/- 10% the reading on the magnehelic gauge. Newer BSCs have displays instead of gauges.

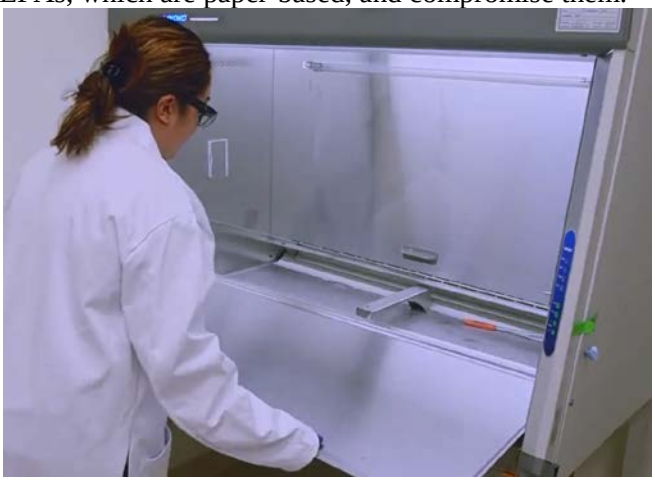


Magnehelic gauge reading	Possible causes/impact
Low	Protective air curtain may be compromised
High	HEPA filter may be loading (compromises airflow)

4. Turn off uv (if present) - reflects off stainless steel and can burn skin and eyes.
5. Operate cabinet blowers for 5 (five) minutes to purge contaminants.

6. Wear personal protective equipment to protect skin and street clothes. Lab coats or solid front gowns should be worn over street clothing and long-cuffed gloves should be worn for hand protection. The cuffs of the gloves should be pulled up and over the cuffs of the coat sleeves. Plastic or Tyvek sleeves should be considered if skin is exposed between the cuff and glove.
7. Adjust your chair so that your armpits are at the same level as the bottom of the sash.
8. Disinfect the work area. Wipe the work surface, interior walls and surface of the window with a suitable disinfectant such as 70% ethanol, an iodophor, or quaternary ammonium compound. Bleach is highly corrosive and should be avoided. Use of bleach without adequate removal of the bleach will pit and rust the stainless steel.

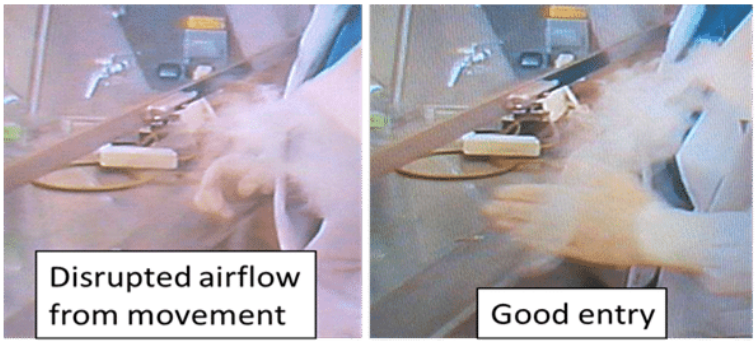
Don't spray the grilles heavily. – that mist can be drawn to the HEPAs, which are paper-based, and compromise them.



The work surface can be removed to clean underneath – wear thick gloves because the edges can be sharp https://www.youtube.com/watch?v=q_C6xq7j-kg Arizona State University (start at 14:10)

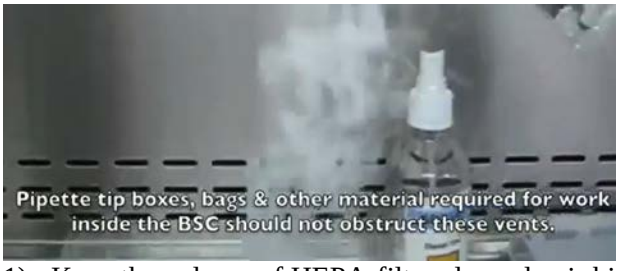
Lint, debris, sharps can all build up underneath the work surface, in the catch basin. https://www.youtube.com/watch?v=fnGy8rCjNu4&list=PLUhbqTxS86maN8AApUJ75P9G-Kf6ZrZYT

9. Set up:
   a. All entries should be perpendicular to the front grille – least airflow disturbance.



   b. Do not block airflow/front and back grilles. It's assumed a person will be in front of the cabinet, but the remainder of the grille area must be clear for airflow.

No protective air curtain. Room air flow directly into the work area (Colorado State) https://www.youtube.com/watch?v=RBJIJzQRhT8



1) Keep the volume of HEPA-filtered supply air high – don't block the grilles.
2) Turbulent air around an obstruction. If a biohazard is in that turbulent air, it's not captured as quickly.

c. Introduce only those items that are required to perform the procedures and arrange them in a logical order. The flow of work should move from clean (media, sterile supplies, etc.) to contaminated areas (waste).

d. Plastic-backed absorbent or a disinfectant-soaked towel can be placed on the work surface to contain any spills or splatters that may occur.

e. Most work should occur at least 4 (four) inches past the front grille.

f. Pipet discard trays containing disinfectant, biohazard bags, sharps containers, etc. should be placed to one side inside the BSC. As mentioned previously, limited motion in and out of the cabinet preserves the protective airflow under the sash.

g. Any equipment generating aerosols, such as a microcentrifuge, vortex or blender, should be placed near the rear of the cabinet (back 1/3 of cabinet).

h. Wipe each item with disinfectant prior to placing it into the cabinet to reduce the introduction of contaminants, (i.e. mold spores and bacteria).

i. For liquid waste, use a vacuum flask containing sufficient disinfectant. Small flasks can be placed inside the BSC. Larger flasks are placed on the floor inside a secondary container to protect against accidental bumping and contain the contents in case the flask breaks. Use an inline hydrophobic 0.22 micron filter to protect the building's vacuum system and Facilities employees.

j. The left picture shows a BSC set up at CDC. Note that work goes from clean (left) to dirty (right) and the pipet disposal tray with lid is inside the cabinet.

- Clean to dirty work flow. Better yet – place the disposal tray on the side, allowing work in the middle to be performed deeper into the cabinet.

10. While working, don't disrupt the protective air curtain

a. When placing arms/hands inside the BSC, wait a few moments to let the airflow stabilize (also may remove surface contaminants from your arms/hands).

b. Introduce hands in perpendicular to the front grille/air curtain.

c. Don't swing arms in and out while working (hint: place pipet tips in an instrument tray inside the cabinet until work is complete)

d. Keep the door closed, and

e. Be aware when someone walks within 6ft behind someone working at the cabinet.

- Hint: tape a one inch wide strip of Kimwipe to the sash edge and watch what happens when the door is opened or someone walks by

11. While working, don't touch the front edge of the cabinet with contaminated gloves (e.g. when pushing away from the cabinet) – that surface touches your lab coat.
12. After work is done, wipe all items with disinfectant and remove them from the cabinet. Do not use the interior of the BSC as a storage area since stray organisms may become "trapped" and contaminate future experiments.
13. Disinfectant the work surface, the sides and back of the cabinet and the back of the sash. Periodically, clean out the catch basin under the work surface.
14. You may keep the BSC on (close the sash, depending on BSC manufacturer) or turn it off. If turning off, let the blowers operate for 5 (five) minutes, with no activity inside the cabinet, to purge the cabinet of any residual biohazardous aerosols.
15. Before leaving the lab, remove lab coats / gowns and gloves and wash your hands.

Visual demonstrations for how to work in a BSC:

CDC online course, Fundamentals of Working in a Biological Safety Cabinet https://www.cdc.gov/labtraining/ (1 hour; there's also a Virtual Reality version)

CDC TRAIN is available to learners across the public health and clinical laboratory community. Learners must set up a free CDC TRAIN account to complete training courses.

NIH National Biosafety and Biocontainment Training Program, Biological Safety Cabinet (BSC): How it Works to Protect You https://www.youtube.com/watch?v=96-aZLom340 (5 minutes)

Effective Use of Class II Biological Safety Cabinets (Digital Training Package), Eagleson Institute https://www.youtube.com/watch?v=DihUzswUkQ8 (3 minute preview)

Working Safely BSC: Proper Preparation, Steps to Take Before You Begin Work (Nuaire) https://www.youtube.com/watch?v=yhNBZ234GiY (5 minutes)

Work from Clean to Contaminated (Nuaire) https://www.youtube.com/watch?v=NgQxOwcOXE0 (3 minutes)

VI. REFERENCES

Baker Company Mythbusters https://bakerco.com/communication/bsc-mythbusters/

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 - Biosafety cabinet selection, installation, lifespan and decommissioning 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 (2019)

VII. APPENDICES/FORMS

Baker Company 'Create a Safe Working Environment in Your Biological Safety Cabinet'

VIII. REVISION HISTORY

Date	Revision No.	Description
11/11/2010	New	Requirements for Class II Biosafety Cabinets
06/27/2018	1	Update title and purpose (BSL2 and above labs), add definitions, harmonize with Lab Safety Training, add video web links, update references, add appendix
09/18/2018	2	Delete redundant text (D.12/13), revise formatting for D.3.
1/12/2022	3	Triennial review, harmonize with 2022 EHS Laboratory Safety Training, update web links, add additional pictures

https://ehs.princeton.edu/laboratory-research/biological-safety/biological-safety-cabinets