ENVIRONMENTAL HEALTH AND SAFETY
University of Nebraska-Lincoln

Safe Operating Procedure

(Revised 6/25)

BIOLOGICAL SAFETY CABINET CLASSIFICATION AND DESIGN

Scope

This SOP describes classification and design of biological safety cabinets (BSCs).
The EHS SOP **Working in a Biological Safety Cabinet (BSC)** provides guidance on the operation and use of biosafety cabinets.

References

Information in this SOP is derived from the following nationally recognized guidelines and standards:

- ***Biosafety in Microbiological and Biomedical Laboratories***, Current edition, Centers for Disease Control and Prevention, National Institutes of Health
- NSF/ ANSI 49 – 2022 Ed., Biosafety Cabinetry: Design, Construction, Performance, and Field Certification

Biological Safety Cabinet Classification

BSCs are designed to provide protection when used with appropriate practices and procedures. Three varieties of BSCs (Class I, II, and III) have been developed to meet varying needs of protection in the laboratory.

1.1 Class I Biological Safety Cabinets

Class I BSCs have one HEPA filter and, when used properly, will protect personnel and the environment from biohazardous material being worked with in the cabinet. However, Class I BSCs cannot protect biological materials being used in the cabinet from environmental microbes and particulates in the room air.

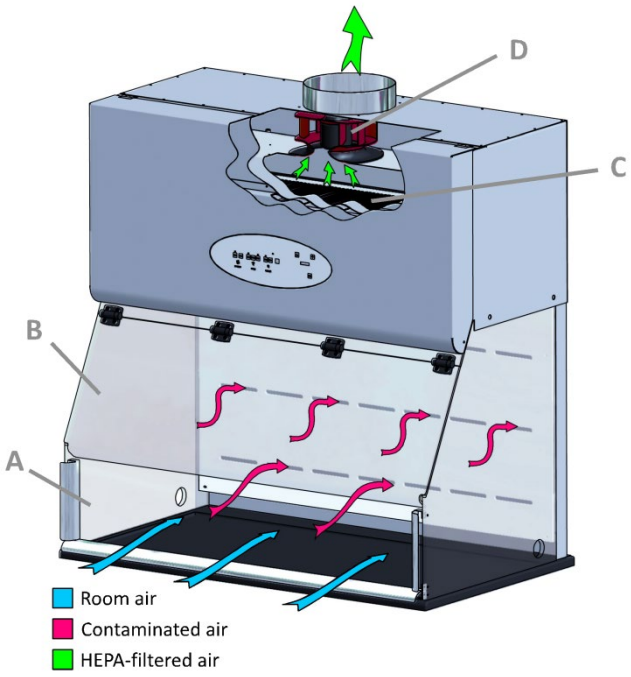
Class I BSCs are partial barrier systems that rely on laminar movement of air to provide containment. If the air curtain is disrupted (e.g., movement of materials in and out of a cabinet, rapid or sweeping movement of the arms), biohazardous materials may be released into the laboratory work environment.

In the Class I BSC, unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward airflow as long as a minimum velocity of 75 linear feet per minute (lfpm) is maintained through the front opening and appropriate work practices are observed.

NOTE: the average person moves between 400-600 lfpm of air when they walk.

Figure 1 Class I Biological Safety Cabinet

Figure



A. front opening, **B.** sash, **C.** exhaust HEPA filter, **D.** blower.

Photo courtesy NuAire, Inc. Laboratory Equipment Supply

1.2 Class II Biological Safety Cabinets (Types, A1, A2 (A/B3), B1, B2 and C1)[1]

Class II BSCs are the predominant design found at UNL and have two HEPA filters located in them. When properly used and maintained, they provide personnel and environmental protection from biohazardous materials being worked with in the BSC and protect the biological product from room contaminants.

[1] NSF International (NSF); American National Standards Institute (ANSI). (2022). Biosafety Cabinetry: Design, Construction, Performance, and Field Certification (NSF/ANSI Standard 49-2022).

These cabinets are also partial barrier systems that rely on laminar movement of air to provide containment. Room air is drawn into the front grille/air vent of the cabinet, providing personnel protection. Air is then directed in a downward laminar flow pattern through a HEPA filter thereby providing product protection. Because the Class II cabinet exhaust air is passed through a HEPA filter, it is particulate-free (environmental protection) and may be recirculated to the laboratory (Type A1 and A2 BSCs) (Figures 2, 3), exhausted from the room via a canopy/thimble connection (Figure 4), or discharged from the BSC to the outside of the building (Types B1 and B2 BSCs) (Figures 5, 6) via a hard duct from the cabinet to the ventilation exhaust system. Type C1 BSCs may or may not be hard ducted to the laboratory room exhaust system.

- Class II A1 and Class II A2s are designed to be recirculating BSCs with about 30% of the air inflow recirculated through the cabinet and 70% of the air inflow exhausted out of the cabinet back into the room environment. The minimum average inflow velocity of a Class II A1 cabinet is 75 lfpm at the face opening of the cabinet. The minimum average inflow velocity of a Class II A2 cabinet is between 100-110 lfpm.

- Class II Type B1 cabinets also recirculate about 30% of the air through the cabinet and exhaust 70% of the air out of the cabinet through the building ventilation exhaust system. Small quantities of volatile chemicals may be worked with in Class II B1 but should be confined to the rear 50% of the cabinet.

- Class II B2 BSCs exhaust 100% of the air through the building ventilation exhaust system allowing some hazardous chemicals to be used in them. Consideration must be given to the chemicals used in this type of BSCs since some chemicals can destroy the filter medium, housing, and/or gaskets causing loss of containment.

- Class II Type C1 BSCs were first introduced by Labconco© in 2014 and added to the NSF 49 Standard as an official type of BSC in 2016. Type C1 BSCs are engineered to have airflow characteristics of both Type A2 and Type B2 biosafety cabinets. Depending on need, the Type C1 may or may not be hard ducted to the laboratory room exhaust system. The airflow diagram is illustrated in Figure 7.

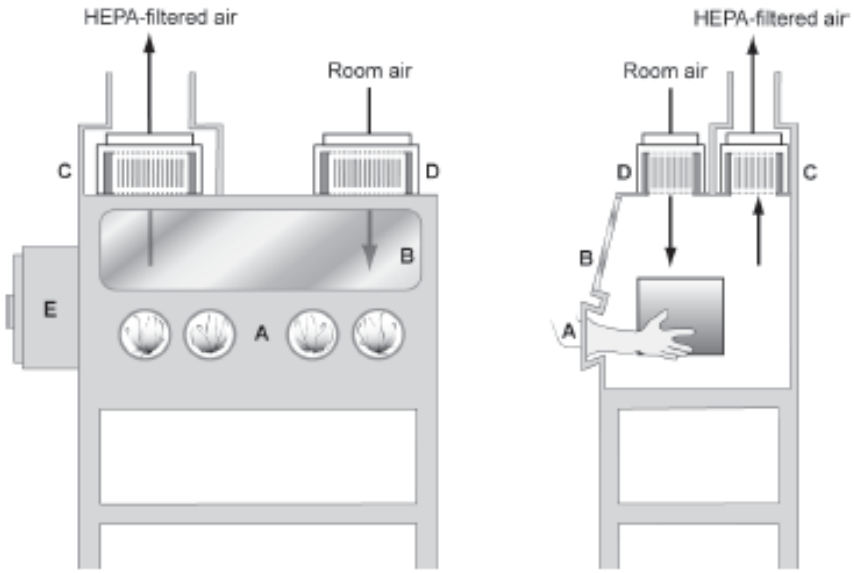
Appendix A shows side views of Class II Type A1 (Figure 2), Class II Type A2 (Figure 3, Canopy (thimble unit for ducting a Class II Type A (Figure 4), side views of Class II Type B1 (Figure 5), Class II Type B2 (Figure 6) and Class II Type C1 Biosafety Cabinets (Figure 7).

1.3 Class III Biological Safety Cabinets

Class III biological safety cabinets (Figure 8) have 3 HEPA filters and offer the maximum protection to laboratory personnel and the environment because all hazardous materials are contained in a completely enclosed, HEPA filtered cabinet. Class III BSCs were designed for work with highly infectious microbiological agents. It is a gas-tight enclosure with a stationary viewing window. Materials are passed into the cabinet through an interlocking door/double-door pass-through box. Both supply and exhaust air are HEPA filtered on a Class III cabinet. Exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge to the outside of the building.

Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow direct manipulation of the materials isolated inside. Due to the design of the cabinet, airflow can be turbulent within the cabinet and laminar airflow is not considered a characteristic of Class III BSCs.

Figure 8 Class III Biological Safety Cabinet



A. glove ports with O-ring for attaching arm-length gloves to cabinet, **B.** sash, **C.** exhaust HEPA filter, **D.** supply HEPA filter, **E.** double-ended autoclave or pass-through box.

Note: A chemical dunk tank may be installed which would be located beneath the work surface of the BSC with access from above. The cabinet exhaust should be hard connected to an independent exhaust system. The exhaust air must be double HEPA filtered, or HEPA filtered and incinerated.

Characteristics & Classifications of Primary Containment Devices

Containment Device	Direction of Airflow (lfpm)	Application/Airflow Pattern	Protection	Appropriate for Some Uses of Volatile Toxic Chemicals and Radionuclides
Laminar Flow Clean Bench	Outward (100)	Applications where the product is not hazardous, but must be kept contaminant free. Provides HEPA filtered supply to the work surface and a particulate-free work area. Appropriate use examples include: plant tissue culture; particulate-free assembly of sterile equipment and electronic devices; polymerase chain reaction (PCR). **(Figures 9, 10)**	Product Only	Not Acceptable
BSC Class I	Inward (≥75)	Air intake is through the front face of the cabinet; exhaust exits through a HEPA to the outside or into the room **(Figure 1)**	Personnel & Environment	Acceptable if hard ducted
BSC Class II, Type A1	Inward (75)	Air intake is through a HEPA filter; 70% of airflow is recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to the outside through a canopy unit. Plenum is under positive pressure. **(Figure 2)**	Product, Personnel & Environment	Minute amounts only if thimble connected to exhaust*
BSC Class II, Type A2 (A/B3 pre-2002)	Inward (100)	Similar to Class II, A1, but has 100 lfpm face velocity and plenums are under negative pressure to room; exhaust air can be ducted to outside through a canopy unit **(Figures 3, 4)**	Product, Personnel & Environment	Minute amounts only if thimble connected to exhaust*
BSC Class II, Type B1	Inward (100)	Air intake is through a HEPA filter; 30-40% of airflow is recirculated in the cabinet and the remaining 60-70% is exhausted through a HEPA filter and a dedicated duct to the outside. **(Figure 5)**	Product, Personnel & Environment	Limited amounts*

BSC Class II, Type B2	Inward (100)	Air intake is through a HEPA filter; 100% of the air is exhausted to the outside via a HEPA-filtered hard duct. No air recirculation in the cabinet. **(Figure 6)**	Product, Personnel & Environment	Acceptable
BSC Class II, Type C1	Inward (100)	This type of cabinet can be operated in different modes depending on whether worker protection from hazardous vapors is a consideration. The mode can be set to approximate either a Type A or Type B biosafety cabinet. **(Figure 7)**	Product, Personnel & Environment	Acceptable, must be hard ducted to lab exhaust and operated in Type B mode.
BSC Class III	Inward	Totally enclosed, gas-tight, glove ports for manipulation of pathogens. Supply air is HEPA filtered. Exhaust air passes through two HEPA filters in series and is exhausted to the outside via a hard connection. Airflow can be turbulent inside the cabinet. **(Figure 8)**	Maximum Product, Personnel, and Environment	Limited amounts*

***In no circumstances should the chemical concentration approach the lower explosion limits of the compound.**

NOTE: Figures 2-7 are in Appendix A

Source: Pan, S. (2025, May 5). *Biosafety Cabinet - Definition, classification (Class I, II, III), working mechanism, application, features. - Biology Notes online*. Biologynotesonline.com. https://biologynotesonline.com/biosafety-cabinet/#google_vignette

Laminar Flow Hood/ “Clean Bench”/ PCR Hood

Laminar flow hoods are **not** biological safety cabinets. They only provide product protection and no protection for the user or for the environment. These cabinets come in two varieties: horizontal and vertical.

Laminar Flow Hoods (Figures 9 and 10) push HEPA-filtered air across the work surface at the user and into the room. They can be used for certain aseptic activities, such as pouring agar plates, filtering media or for polymerase chain reactions (PCR). These clean benches should **never** be used when: handling or manipulating potentially infectious materials, for any cell culture materials, toxins, hazardous chemicals or drug formulations. The worker and the lab room will be exposed to the materials being manipulated in the laminar flow hood.

Figure 9 Horizontal laminar flow hood/ “clean bench”

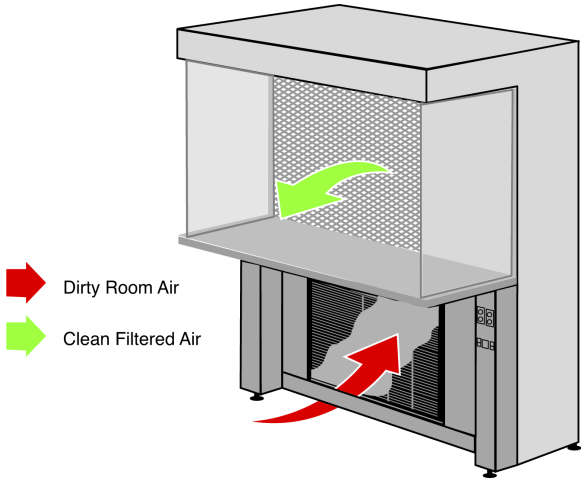


Photo courtesy NuAire, Inc. Laboratory Equipment Supply

A. front opening, **B.** supply grille, **C.** supply HEPA filter, **D.** supply plenum, **E.** blower, **F.** grille.

Figure 10 Vertical laminar flow hood/ “clean bench”

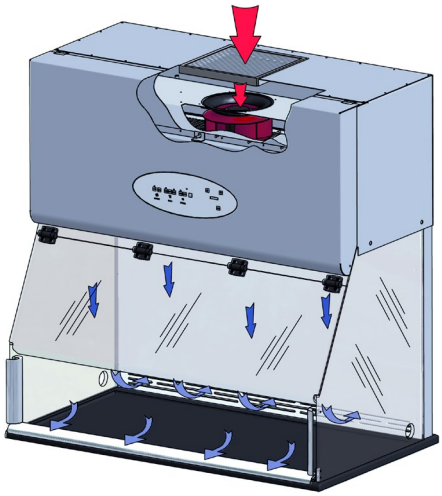


Photo courtesy NuAire, Inc. Laboratory Equipment Supply

A. front opening, **B.** sash, **C.** supply HEPA filter, **D.** blower.

KEY: Red arrows denote Room Air. Green Arrows denote Clean HEPA filtered air.

Site Assessment and Proper BSC Placement

Principal Investigators should consult with the Biosafety Officer (BSO) and request a risk assessment of the proposed research to ensure that an appropriate BSC is used for the work.

The cabinet should be located away from traffic patterns, doors, fans, supply air vents, fume hoods and any other air-handling device that could disrupt its airflow patterns. All windows that can be opened in the room should remain closed. Figure 11 shows the preferred location for the cabinet. The BSC should be located at the wall furthest from and facing the entry door. If this is not possible, the BSC should be located on the side wall perpendicular to the hinge side of the door.

BSCs not connected to an exhaust system should have at least 12-18 inches of clearance from the exhaust filter on top of the cabinet and any overhead obstructions when the cabinet is in its final operating position, to allow for testing of the Exhaust HEPA filter and for proper fire sprinkler system coverage.

All BSCs should be placed in a laboratory at a location that provides a minimum of:

- ❑ 6 inches from adjacent walls or columns
- ❑ 6 inches between two BSCs
- ❑ 6 inches space between both sides of the cabinet and 6 inches behind the BSC to allow for service operations
- ❑ 40 inches of open space in front of the BSC
- ❑ 60 inches from opposing walls, bench tops and areas of occasional traffic
- ❑ 20 inches between BSC and bench tops along a perpendicular wall
- ❑ 100 inches between two BSCs facing each other
- ❑ 60 inches from behind a doorway
- ❑ 40 inches from an adjacent doorway swing side and
- ❑ 6 inches from an adjacent doorway (hinge side)

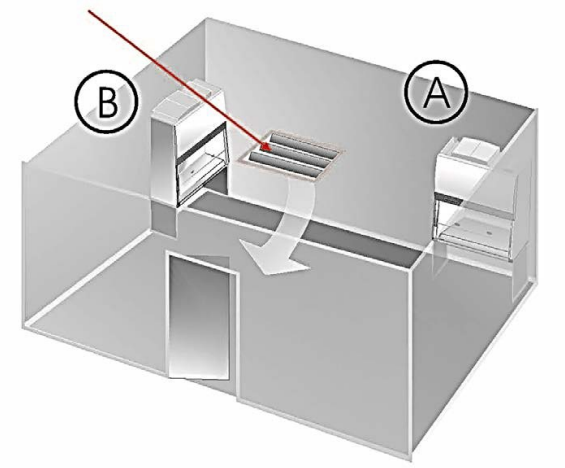
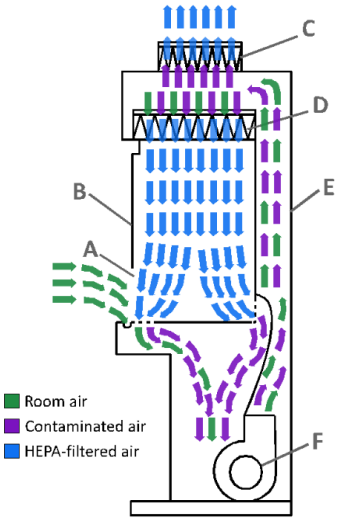


Figure 11. Location A shows the preferred location. Location B is an alternate location. The air supply register(s) above or near the cabinet's location should be redirected away from the cabinet face. (Image from NSF/ANSI 49-2018 Annex E)

Appendix A

Figures 2 – 7: Side view of Class II Biosafety Cabinet Types

Figure 2
Class II Type A1 BSC



Side View

A. front opening, **B.** sash, **C.** exhaust HEPA filter,
D. supply HEPA filter, **E.** common plenum, **F.** blower.

Figure 3 Side View

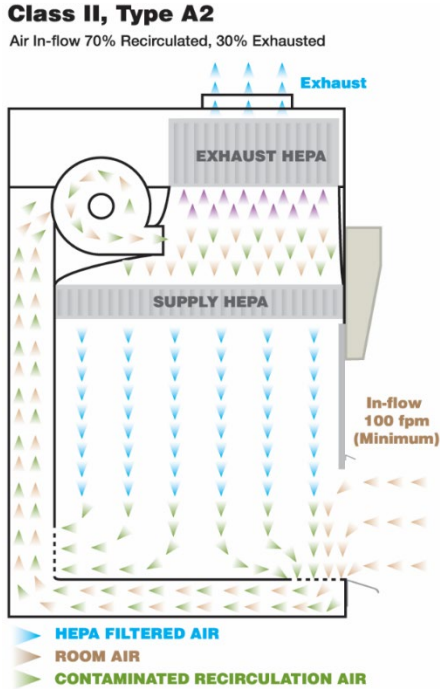
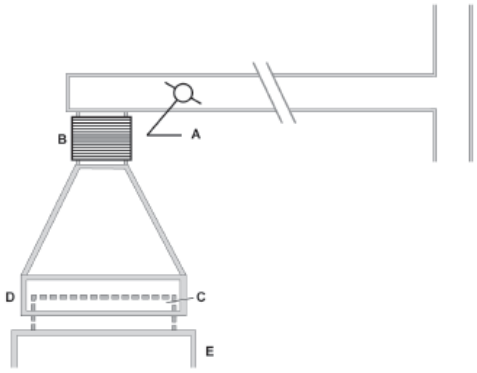


Figure 4 Canopy (thimble) unit for ducting a Class II, Type A BSC



A. balancing damper, **B.** flexible connector to exhaust system, **C.** cabinet exhaust HEPA filter

Note: There is a 1" gap between the canopy unit (D) and the exhaust filter housing (C), through which room air is exhausted.

Figure 5 (Side View)

Class II, Type B1

Air In-flow 30% Recirculated, 70% Exhausted

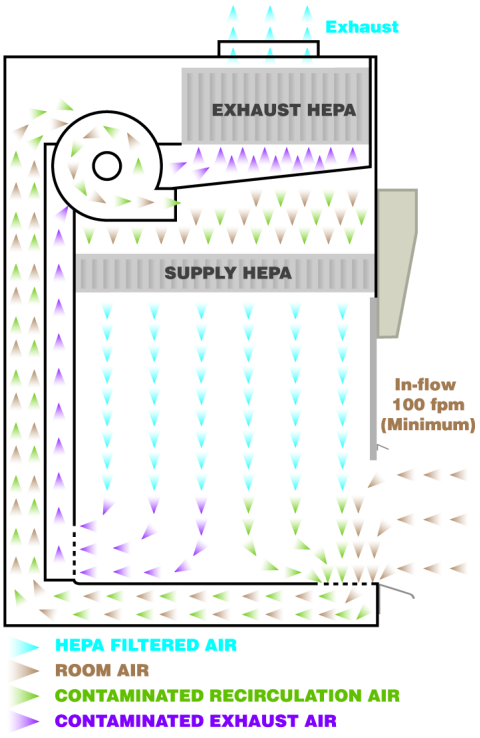
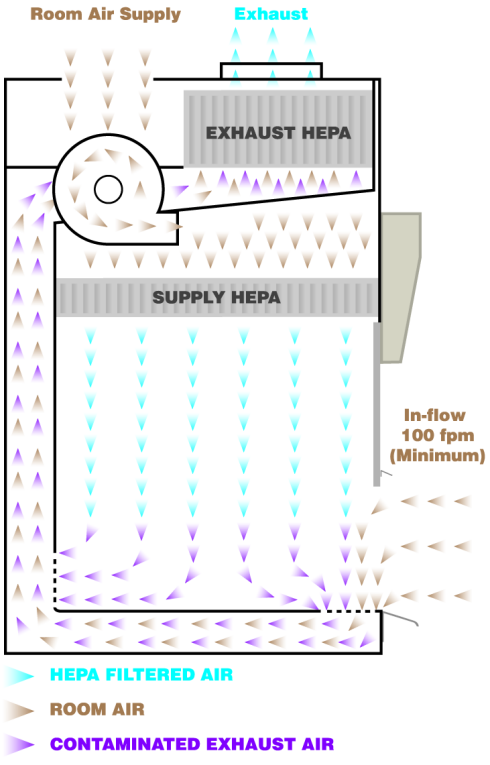


Figure 6 (Side View)

Class II, Type B2 Air In-flow 100% Exhausted



Photos courtesy NuAire, Inc. Laboratory Equipment Supply

Figure 7

Class II, Type C1 Biosafety Cabinet

Photo courtesy Labconco Corporation