



BIOSAFETY CABINETS

Scope

This SOP describes the classification and design of biological safety cabinets. Guidance on operation and use procedures of biosafety cabinets is provided in the EHS SOP ***Working in a Biosafety Cabinet***.

References

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

- **Biosafety in Microbiological and Biomedical Laboratories**, (BMBL, 6th Ed., Centers for Disease Control and Prevention, National Institutes of Health
- NSF/ ANSI 49 – 2022 Ed., Biosafety Cabinetry: Design, Construction, Performance, and Field Certification

Biosafety Cabinet Classification

Biological Safety Cabinets (BSCs) are an engineering control designed to provide personnel, product, and environmental protection when used with appropriate practices and procedures. Three varieties of BSCs (Class I, II, and III) have been developed to meet varying needs in the laboratory. Class II cabinets are the predominant design found at UNL.

Use of Class I BSCs should be limited to basic microbiological work with low- and moderate-risk agents. Class II BSCs, when properly maintained and used with good microbiological techniques, provide an effective containment system for safe manipulation of moderate and high-risk microorganisms (risk group (RG) 2 and 3 agents).



NOTE: All work with RG-2 and RG-3 organisms and recombinant or synthetic nucleic acids requires UNL IBC review and approval prior to initiation of experiments. Consult the **UNL Biosafety Guidelines** for additional information.

The inward face velocities of Class I and II BSCs (75-100 linear feet per minute) provide comparable levels of containment to protect laboratory workers and the immediate environment from infectious aerosols generated within the cabinet. Class II BSCs also provide product protection through high-efficiency particulate air filtration (HEPA filtration) of the intake air. Class III cabinets offer the maximum protection to laboratory personnel, the community,

and the environment because all hazardous materials are contained in a completely enclosed, ventilated cabinet.

All classes of BSCs are equipped with exhaust HEPA filters, which distinguishes them from laminar flow “clean benches.” Additionally, Class II and III BSCs along with “clean benches” protect the products/cultures within from environmental contamination by using a supply air HEPA filter. In contrast, Class I BSCs are not equipped with supply HEPA filters and as such provide no product protection. Design differences between various classes of biosafety cabinets are summarized in Table 1 below.



NOTE: A “clean bench,” although providing either vertical or horizontal laminar airflow, is **not** a BSC and users should be aware of the limitations of these devices. Their design and use is discussed later in this document (Figures 9, 10).

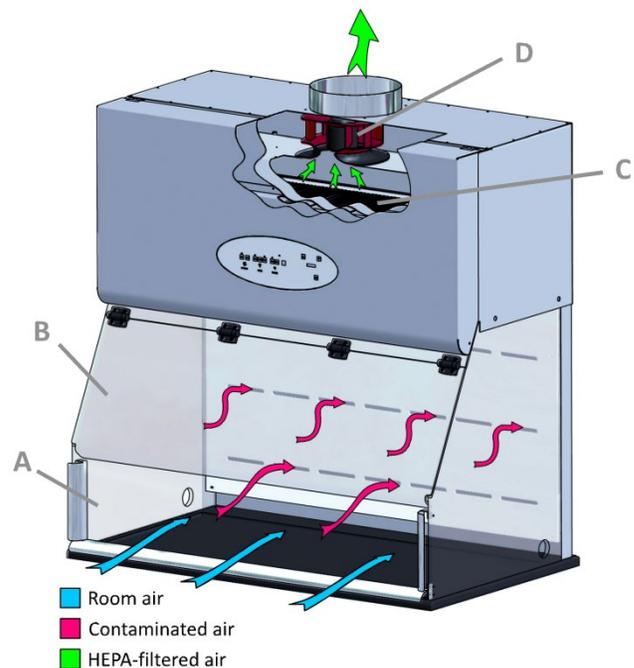
Class I BSC

(Note: Class I BSCs are manufactured on a limited basis. Most have been replaced with Class II BSCs.)

The Class I BSC provides personnel and environmental protection, but no product protection. Air movement is similar to a chemical fume hood, but it has a HEPA filter in the exhaust system to protect the environment (Figure 1) from bioaerosols. 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.

The classical Class I BSC is hard-ducted (i.e., direct connection) to the building exhaust system, and the building exhaust fan provides the negative pressure necessary to draw room air into the cabinet.

Figure 1 Class I Biological Safety Cabinet



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

Note: The cabinet needs to be hard connected to the building exhaust system if toxic vapors are to be used.

Photo courtesy [NuAire, Inc. Laboratory Equipment Supply](#)

Table 1. Characteristics and Classifications of Primary Containment Devices^{1,2}

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 and 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, and 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, and 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, and 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, and 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, and 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.*

Class II BSC

Class II 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) potential for contaminant release into the laboratory work environment is increased as is the risk of product contamination.

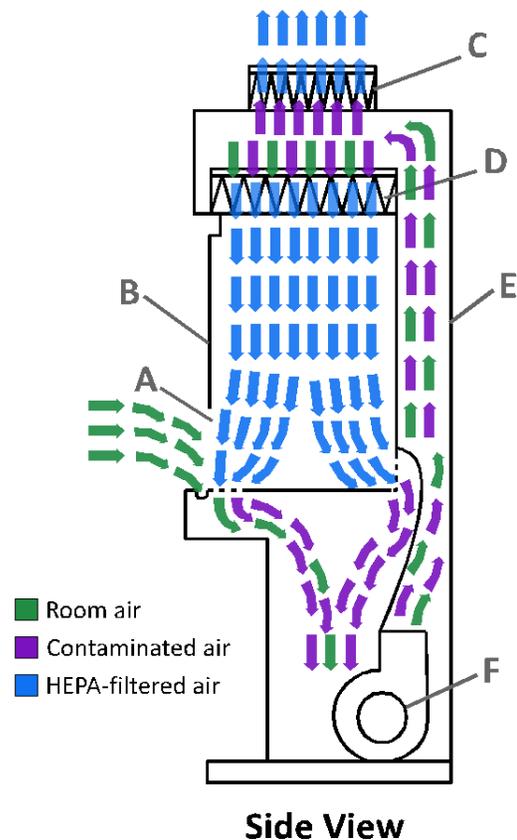
Class II (Types A1, A2, B1, B2 and C1)¹ BSCs provide personnel, environment, and product protection. Airflow is drawn into the front grille of the cabinet, providing personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination across the work surface of the cabinet. Because 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) or discharged from the building via a canopy connection (Figure 4). Exhaust air from Types B1 and B2 BSCs (Figures 5, 6) must be discharged to the outdoors via a hard connection.

An internal blower (Figure 2) draws sufficient room air through the front grille to maintain a minimum average inflow velocity of at least 75 lfpm at the face opening of the cabinet. The supply air flows through a HEPA filter and provides particulate-free air to the work surface. Laminar (unidirectional, constant velocity) airflow reduces turbulence in the work zone and minimizes the potential for cross-contamination.

The downward moving air "splits" as it approaches the work surface; the blower draws part of the air to the front grille and the rest is drawn to the rear grille. Although there are variations among different cabinets, this split generally occurs about halfway between the front and rear grilles and 2-6 inches above the work surface.

It is possible to exhaust the air from a Type A1 or A2 cabinet outside of the building. However, it must be done so as not to alter the balance of the cabinet exhaust system, and thereby disturbing the internal cabinet airflow. The proper method of connecting a Type A1 or A2 cabinet to

Figure 2
Class II Type A1 Biological Safety Cabinet



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

the building exhaust system is through use of a canopy hood,¹ which provides a small opening (usually 1 inch) around the cabinet exhaust filter housing (Figure 4). The current NSF/ANSI 49 Standard requires Class II Type A1 or A2 cabinets with canopy connections be fitted with an audible and visible exhaust alarm to indicate when air flow is being returned to the room rather than being exhausted out. Annual certification of cabinets with these “thimble connections” must include alarm function certification.

Figure 3
Class II, Type A2

Air In-flow 70% Recirculated, 30% Exhausted

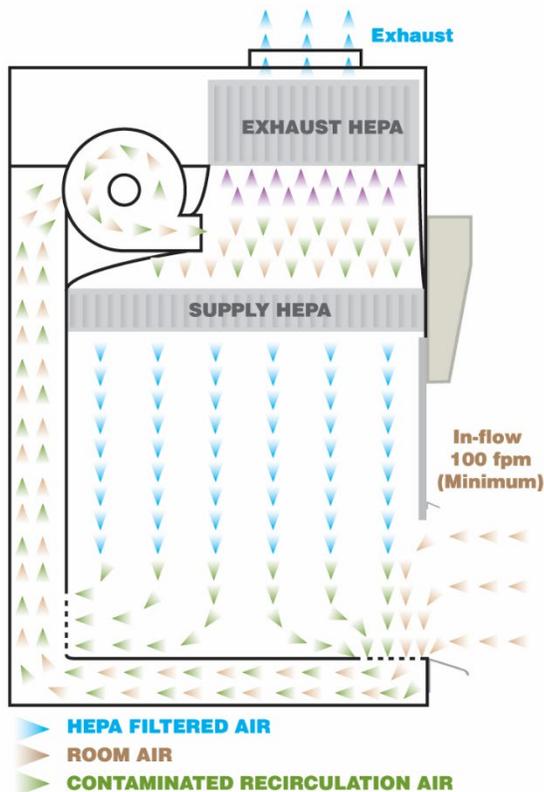
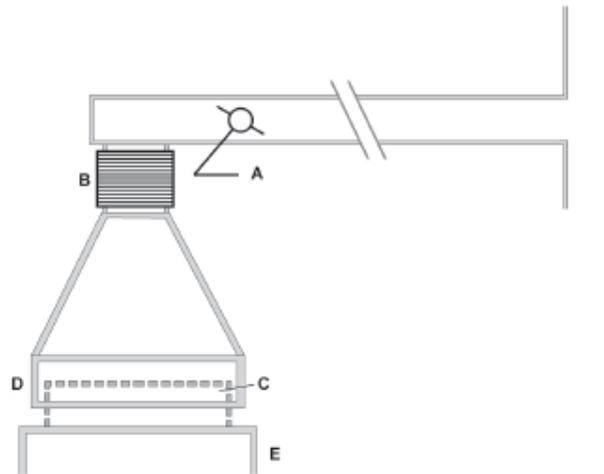


Photo courtesy

[NuAire, Inc. Laboratory Equipment Supply](http://www.nuaire.com)

Figure 4 Canopy (thimble) unit for ducting a Class II, Type A BSC²



A. balancing damper, **B.** flexible connector to exhaust system, **C.** cabinet exhaust HEPA filter housing, **D.** canopy unit, **E.** BSC.

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

In a Class II, Type B1 cabinet, the supply blowers in the cabinet draw room air (plus a percentage of the cabinet’s recirculated air) through the front grille. This air is taken up through plenums in the side of the cabinet, 30% is forced through the supply HEPA filter, and 70% is drawn through the exhaust HEPA by the building exhaust system. Approximately 70% of the down flow supply air is drawn into the rear grille and is directly exhausted through a HEPA to the building exhaust. Since air near the rear of the cabinet is directly exhausted and not recirculated, small quantities of volatile chemicals may be worked with in the cabinet, but should be confined to the rear 50% of the cabinet.

Figure 5

Class II, Type B1

Air In-flow 30% Recirculated, 70% Exhausted

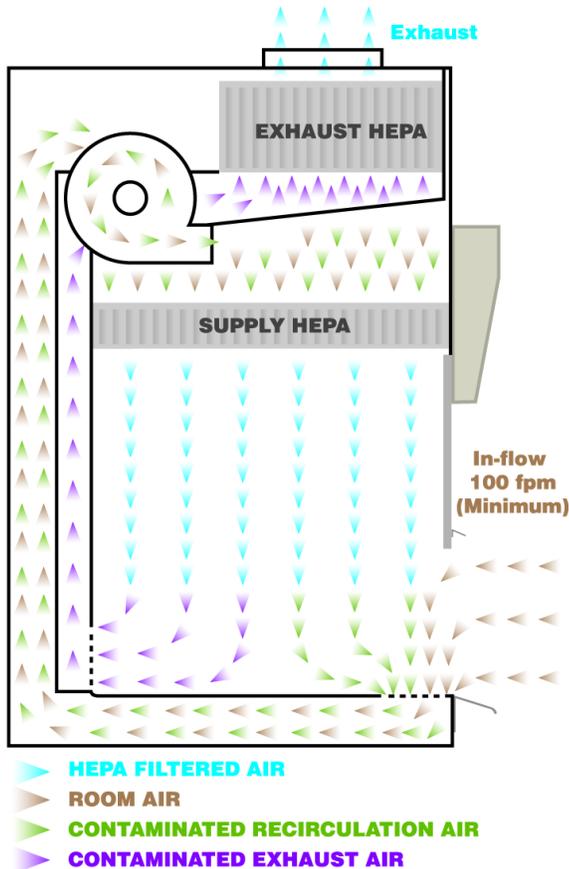


Photo courtesy

[NuAire, Inc. Laboratory Equipment Supply](http://www.nuaire.com)

Note: The cabinet exhaust needs to be hard connected to the building exhaust system.

Figure 6

Class II, Type B2

Air In-flow 100% Exhausted

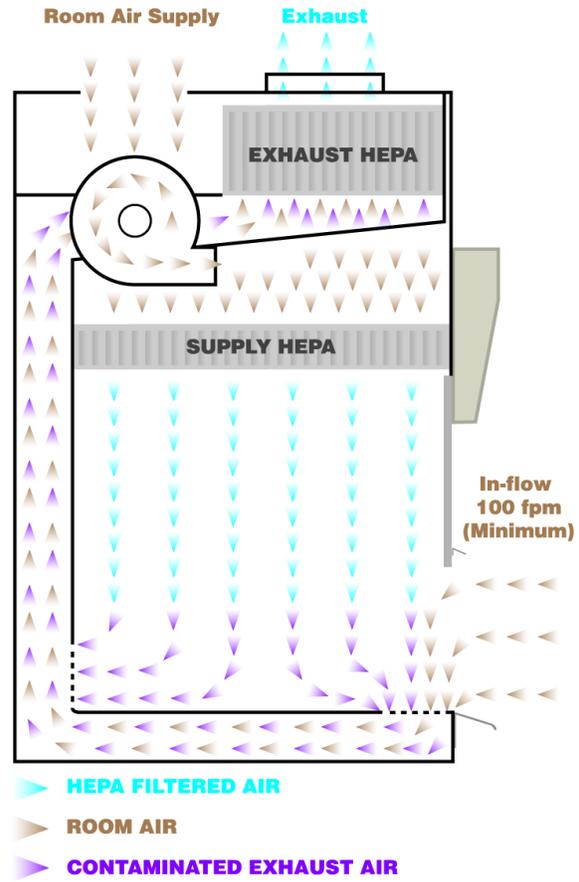


Photo courtesy

[NuAire, Inc. Laboratory Equipment Supply](http://www.nuaire.com)

Note: The cabinet exhaust needs to be hard connected to the building exhaust system.

Class II, Type B2, BSCs (Figure 6) are total exhaust cabinets; no air is recirculated. This cabinet provides both biological and chemical containment. Consideration must be given to the chemicals used in BSCs since some chemicals can destroy the filter medium, housings, and/or gaskets causing loss of containment. The supply blower draws either room or outside air in at the top of the cabinet, passes it through a HEPA filter and down into the work area of the cabinet. The building exhaust system draws air through both the rear and front grills producing an inflow face velocity of 100 lfpm. All air entering this cabinet is exhausted through a HEPA filter.

Class II Type C1 BSC

First introduced by Labconco® in 2014 and added to the NSF 49 Standard as an official type of BSC in 2016, the Type C1 is engineered to have airflow characteristics of both Type A2 and Type B2 biosafety cabinets. In addition, this design provides a unique workspace to ensure optimal airflow and worker/product protection. Depending on need, the Type C1 may or may not be hard-ducted to laboratory exhaust. The airflow diagram is illustrated in Figure 7.

The Class II Type C1 has two motor/blowers. The supply motor/blower is positioned above the supply HEPA filter, and is responsible for the recirculation of air from the front grille and sides of the work area back down through the work area. The exhaust motor/blower is located before the exhaust HEPA filter, and it draws air from the center of the work area, and pushes it through the filter, discharging HEPA-filtered air either back into the laboratory, or into an exhaust system. The exhaust design resembles a Type A2, while the airflow pattern resembles a Class II Type B1 design. This new design is referenced in the NSF/ANSI 49-2018 revision.

Class III BSC

The Class III BSC (Figure 8) was designed for work with highly infectious microbiological agents and for the conduct of hazardous operations and provides maximum protection for the environment and the worker. It is typically appropriate for BSL-4 containment. It is a gas-tight enclosure with a stationary viewing window. Materials are passed into the cabinet through a dunk tank, that is accessible through the cabinet floor, or double-door pass-through box (e.g., autoclave) that can be decontaminated between uses. The same process is reversed to take items out of the cabinet. Both supply and exhaust air are HEPA filtered on a Class III cabinet and the exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge to the outside.

Figure 7
Class II, Type C1 Biosafety Cabinet

Photo courtesy [Labconco Corporation](http://www.labconco.com)

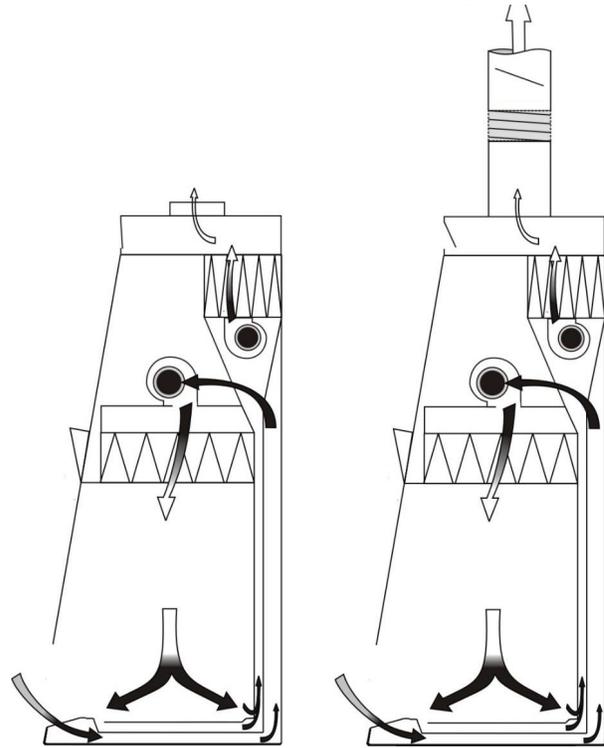
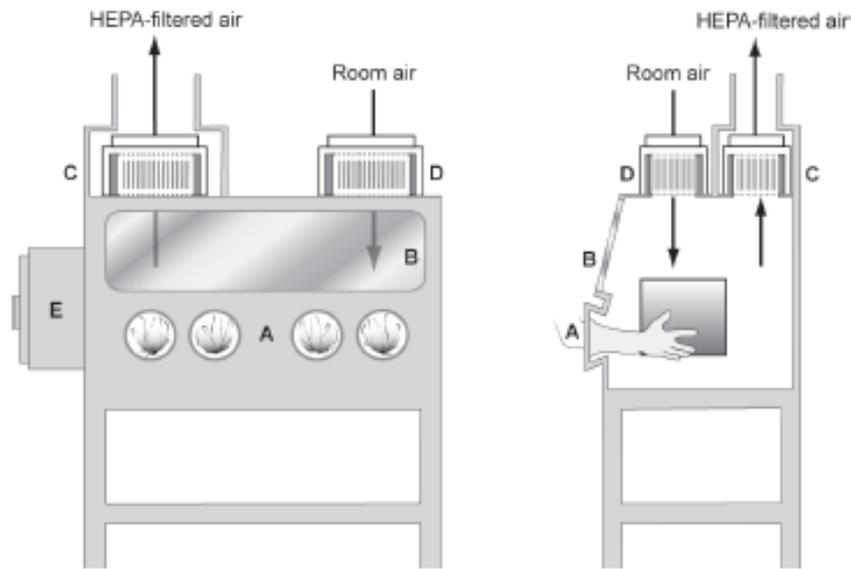


Figure 8 Class III Biological Safety Cabinet



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 needs to be hard connected to an independent dedicated exhaust system. The exhaust air must be double HEPA filtered or HEPA filtered and incinerated.

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 a Class III BSC.

Laminar Flow “Clean Bench”

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

Clean benches (Figures 9 and 10) discharge HEPA-filtered air across the work surface and toward the user. ***These devices do not provide personnel or environmental protection.*** They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices. Clean benches should never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials. The worker will be exposed to the materials being manipulated on the clean bench.

Figure 9 Horizontal laminar flow “clean bench”

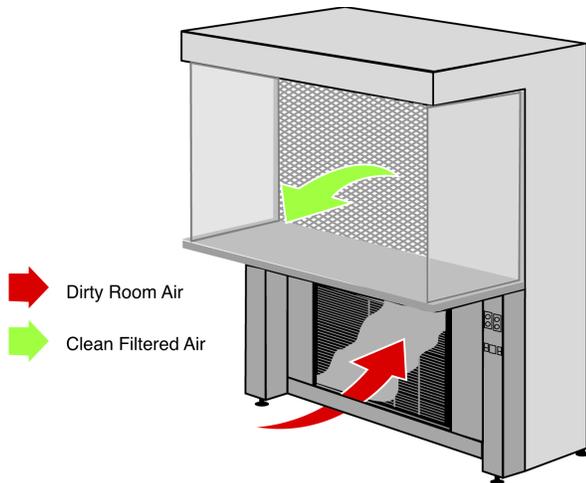


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A. front opening, **B.** supply grille, **C.** supply HEPA filter, **D.** supply plenum, **E.** blower, **F.** grille.

Figure 10 Vertical laminar flow “clean bench”

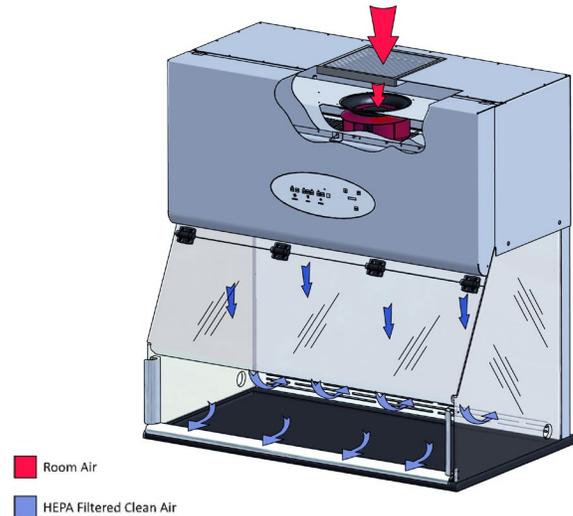


Photo courtesy

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

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, ventilation registers, fume hoods and any other air-handling device that could disrupt its airflow patterns. All windows in the room should be closed. Figure 8 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 inches) 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/ULPA filter.

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

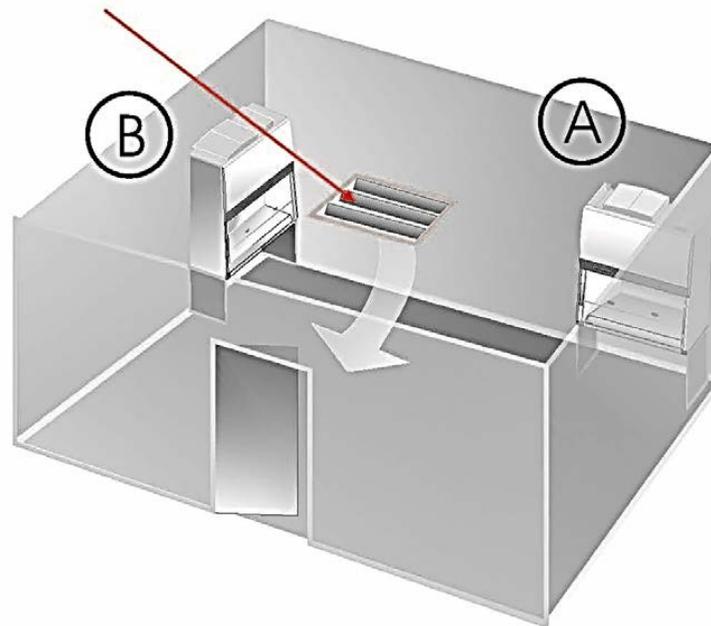


Figure 11 Location A shows the preferred location. Location B is an alternate location. The air supply register(s) above or near the cabinet's location should be redirected away from the cabinet face. (Image from NSF/ANSI 49-2018 Annex E)

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

²Centers for Disease Control and Prevention, N. I., Biosafety in Microbiological and Biomedical Laboratories (6th edition). Washington, DC: U.S. Government Printing Office.