

## CHAPTER 16

### BIOLOGICAL SAFETY CABINETS

#### Overview

This chapter describes the containment principles of biological safety cabinets (BSCs), the various classes and types of BSCs and their uses, how to select the correct type of BSC for your needs, how to get approval for installation of a BSC, and their installation and certification requirements.

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## CHAPTER 16

### BIOLOGICAL SAFETY CABINETS

#### I. Introduction

Laboratory techniques may produce aerosols, which can contain hazardous research materials, such as infectious agents that laboratory workers could inhale. Biological safety cabinets (BSC) are one type of primary barrier to contain potentially infectious research materials in order to prevent exposure of laboratory personnel and contamination of the general environment. Some biological safety cabinets also provide a clean work environment to protect cell cultures or sterile apparatus. Discussion of another type of primary barrier, the laboratory hood, is in Chapter 17.

#### II. Principles of Containment

Containment of hazardous aerosols in biological safety cabinets occurs by air barriers, physical barriers, and high-efficiency particulate air (HEPA) filtration. Air barriers provide containment by providing directional airflow from the laboratory, past the researcher, and into the cabinet via the work opening. Hazardous aerosols generated during experimental procedures inside the cabinet become captured and carried by the flow of air, and trap within HEPA filters. Some BSCs provide protection for experimental procedures by providing uniform, unidirectional HEPA filtered air, referred to as laminar airflow, continuously flowing over the work area. Laminar airflow minimizes turbulence inside the cabinet, allowing for immediate removal of contaminants generated by the procedures. Disruption of the airflow patterns in the cabinet can compromise the integrity of the containment provided by air barriers, and objects within the cabinet can disrupt airflow patterns. Therefore, air barriers provide only partial containment, and you should not use them solely to contain highly toxic or infectious materials.

Physical barriers are impervious surfaces such as metal sides, glass panels, rubber gloves and gaskets, which physically separate the experimental procedures from the researcher. Biological safety cabinets incorporating physical barriers (e.g. Class III BSCs), and not relying on air barriers, can be used for higher risk agents since compromised containment is less likely.

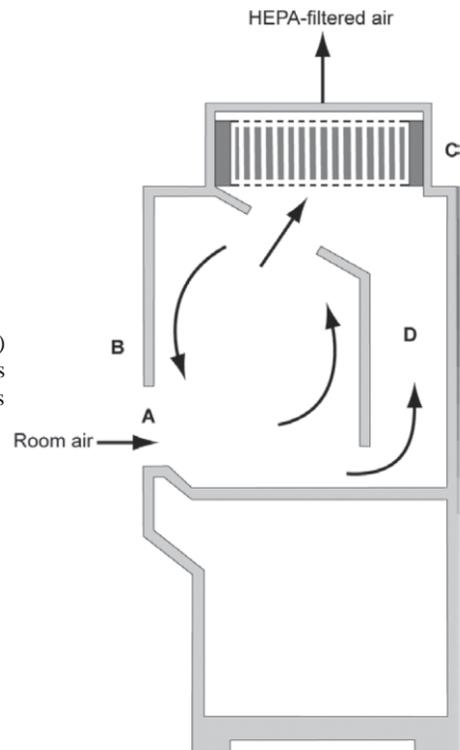
HEPA filters have a filtration efficiency of 99.97% for thermally generated monodisperse dioctylphthalate (DOP) 0.3  $\mu\text{m}$  diameter particles. Because of their high efficiency, HEPA filters in biological safety cabinets can remove virtually all particulates, including hazardous microbiological and chemical aerosols, in the air stream passing through the filter. All biological safety cabinets have exhaust filters that remove contaminants as air discharges from the cabinet. Some types (discussed below) also have supply HEPA filters to provide clean air to the work area. HEPA filters are not effective in capturing chemical vapors, and are not considered protective against gases or vapor-phase solids/liquids.

### III. Classification of Biological Safety Cabinets

There are three classes of biological safety cabinets, designated as Class I, Class II, and Class III. Class I and II cabinets have a protective air barrier across the work opening that separates the laboratory researcher from the work area. Class II cabinets also provide a HEPA-filtered, clean work area to protect the experiment from room contamination. Several variations of Class II cabinets are described below. Class III cabinets have a physical barrier between the operator and the work area. Arm length rubber gloves sealed to glove ports on the cabinet provide the operator with access to the work area. The distinctive features of the three classes of cabinets follow. Refer to Table 16-1 for a comparison of the characteristics and applications for the types of BSCs described below, or view this [Centers for Disease Control and Prevention document](#).

#### A. Class I Cabinets (Figure 16-1)

- The Class I cabinet is ventilated for personnel and environmental protection, with an inward airflow away from the operator. It is similar in air movement to a laboratory hood.
- The minimum average face velocity through the work opening is 75 feet per minute (fpm).
- The cabinet exhausts air through a HEPA filter to prevent discharge of most particles to the outside atmosphere.
- This cabinet is suitable for work with low and moderate risk biological agents, where no product protection is required.
- Because of the popularity of Class II cabinets and the product protection they provide, use of Class I cabinets has declined.



**Figure 16.1** *The Class I BSC* (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) exhaust plenum. *Note:* The cabinet needs to be hard connected to the building exhaust system if toxic vapors are to be used.

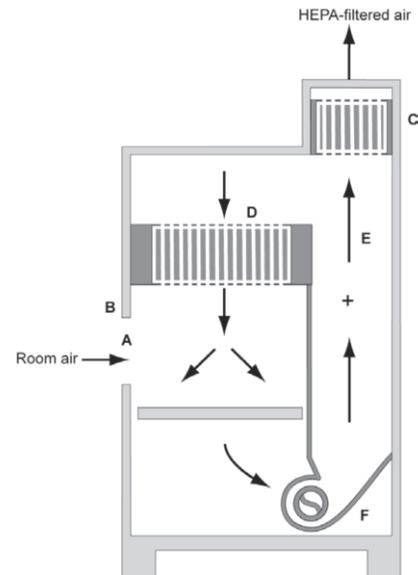
## B. Class II Cabinets

- The Class II cabinet ventilates air for personnel, product, and environmental protection, and has an open front and inward airflow for personnel protection.
- Product protection comes from HEPA filtered laminar airflow from a diffuser located above the work area. The downflow air splits at the work surface, and exits the work area through grilles located at both the rear and front of the work surface, respectively.
- The cabinet has HEPA filtered exhausted air for environmental protection.
- Types of Class II biological safety cabinets are designated A1, A2, B1, and B2.

### 1. Class II, Type A1 (Formerly Type A) Cabinets (Figure 16-2)

- The work opening is 8 to 10 inches (20-25 cm) high.
- The type A cabinet may have a fixed work opening, a sliding sash, or a hinged window.
- A fan located within the unit provides the intake, recirculated supply air, and the exhaust air.
- The BSC fan maintains a minimum average inflow velocity of 75 fpm through the work area access opening.
- Approximately 70% of the cabinet air recirculates through a HEPA filter into the work area from a common plenum, while approximately 30% of the air enters through the front opening and an amount equal to the inflow is exhausted from the cabinet through a HEPA filter.
- The cabinet may exhaust HEPA filtered air back into the laboratory or exhaust to the environment through an exhaust canopy.
- The cabinets may have positive pressure contaminated plenums. Contaminated plenums under positive pressure must be gas tight.
- Type A1 cabinets are suitable for work with low to moderate risk biological agents in the absence of volatile toxic chemicals and volatile radionuclides.

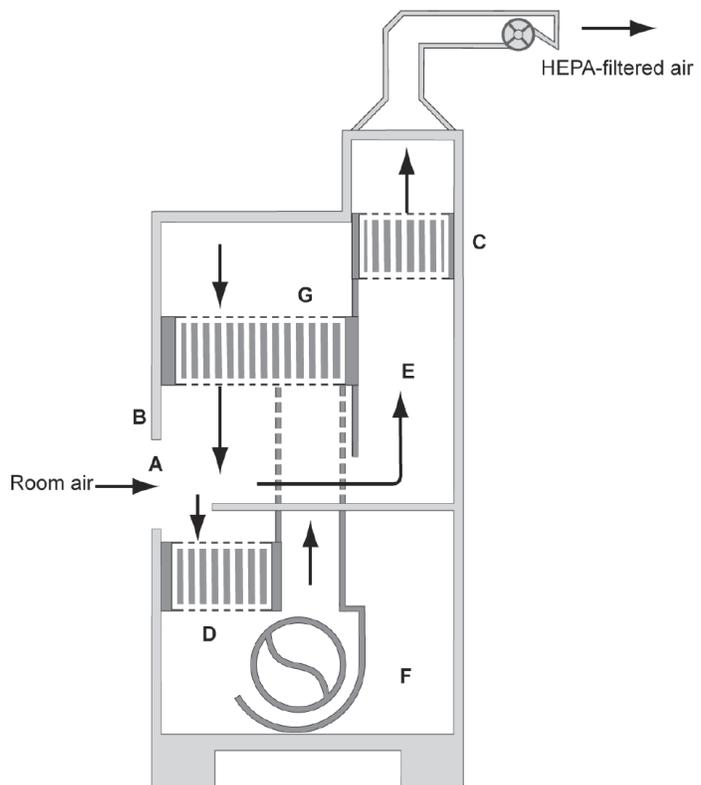
**Figure 16.2** *The Class II, Type A1 BSC* (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) common plenum; (F) blower.



## 2. Class II, Type B1 Cabinets (Figure 16-3)

- The work opening is 8 inches (20 cm) high, with a sliding sash that one can raise for introduction of equipment into the cabinet.
- Type B1 cabinets have a minimum average inflow velocity of 100 fpm through the work area access opening.
- The HEPA filtered downflow air is composed largely of uncontaminated recirculated inflow air.
- Supply fans located in the base of the cabinet, below the work surface, draw air through a grille at the front of the work surface, and supply HEPA filters located directly below the work surface. The fans then force the filtered air through plenums in the sides or the rear of the cabinet and recirculate the air through a diffuser above the work surface. Some cabinets have a secondary supply filter located above the work surface.
- Approximately 70% of the contaminated downflow air is exhausted through a HEPA filter and a dedicated duct and then discharged outside the building.
- The remote exhaust fan is generally located on the roof of the building.
- All biologically contaminated ducts and plenums are under negative pressure or surrounded by negative pressure ducts and plenums. The type B1 cabinet is suitable for work with low to moderate risk biological agents. They are also useful for biological materials treated with minute quantities of toxic chemicals and trace amounts of radionuclides.

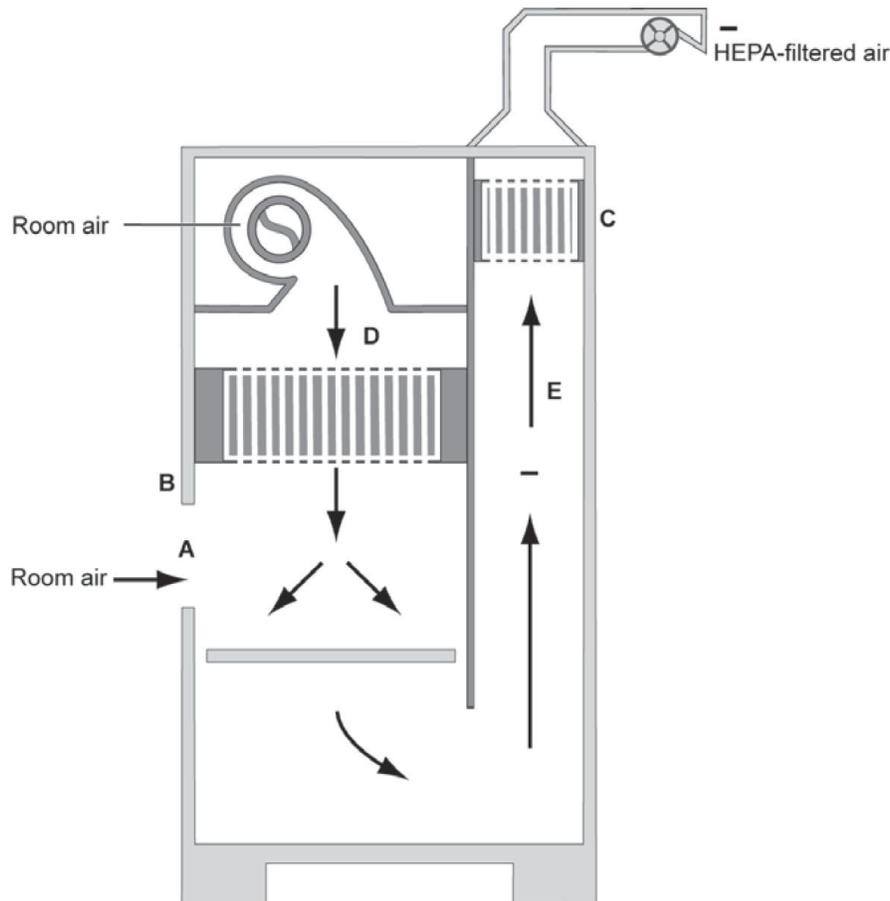
**Figure 16.3** The Class II, Type B1 BSC (classic design) (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure dedicated exhaust plenum; (F) blower; (G) additional HEPA filter for supply air. *Note:* The cabinet exhaust needs to be hard connected to the building exhaust system.



### 3. Class II, Type B2 ("Total Exhaust") Cabinets (Figure 16-4)

- The type B2 cabinet has a sliding sash with an 8-inch (20 cm) opening.
- The type B2 cabinet maintains a minimum average inflow velocity of 100 fpm through the work area access opening.
- No air recirculates within the cabinet.
- A supply fan draws air from the laboratory and forces it through a supply HEPA filter located over the work area.
- A remote exhaust fan, generally located on the roof, pulls all inflow air and supply air through a HEPA filter, and discharges it outside the building. As much as 1200 cubic feet per minute may be exhausted from a 6 ft. cabinet.
- The cabinet has all contaminated ducts and plenums under negative pressure or surrounded by directly exhausted (not recirculated through work area) negative pressure ducts and plenums.
- Type B2 cabinets are suitable for work with low to moderate risk biological agents. They are also useful for biological materials treated with toxic chemicals and radionuclides.

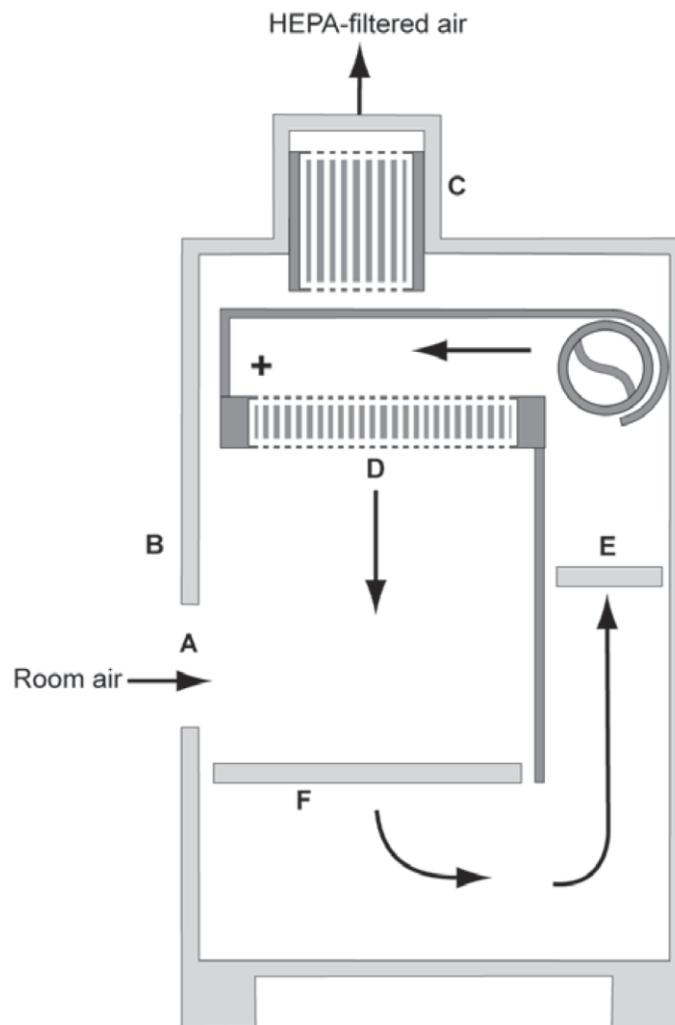
**Figure 16.4** The Class II, Type B2 BSC (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure exhaust plenum. *Note:* The carbon filter in the exhaust system is not shown. The cabinet needs to be hard connected to the building exhaust system.



#### 4. Class II, Type A2 (Formerly B3) Cabinets (Figure 16-5)

- Type A2 cabinets have a minimum of 100 fpm average inflow velocity.
  - All biologically contaminated ducts and plenums are under negative pressure or surrounded by negative pressure ducts and plenums
  - They may exhaust HEPA filtered air back into the laboratory or to the environment through an exhaust canopy.
- Type A2 cabinets are suitable for work with low to moderate risk biological agents. Type A2 cabinets used for work with minute quantities of volatile toxic chemical and trace amounts of radionuclides must be exhausted through properly functioning exhaust canopies. If the cabinet is not ducted, you cannot work with chemicals in the cabinet.

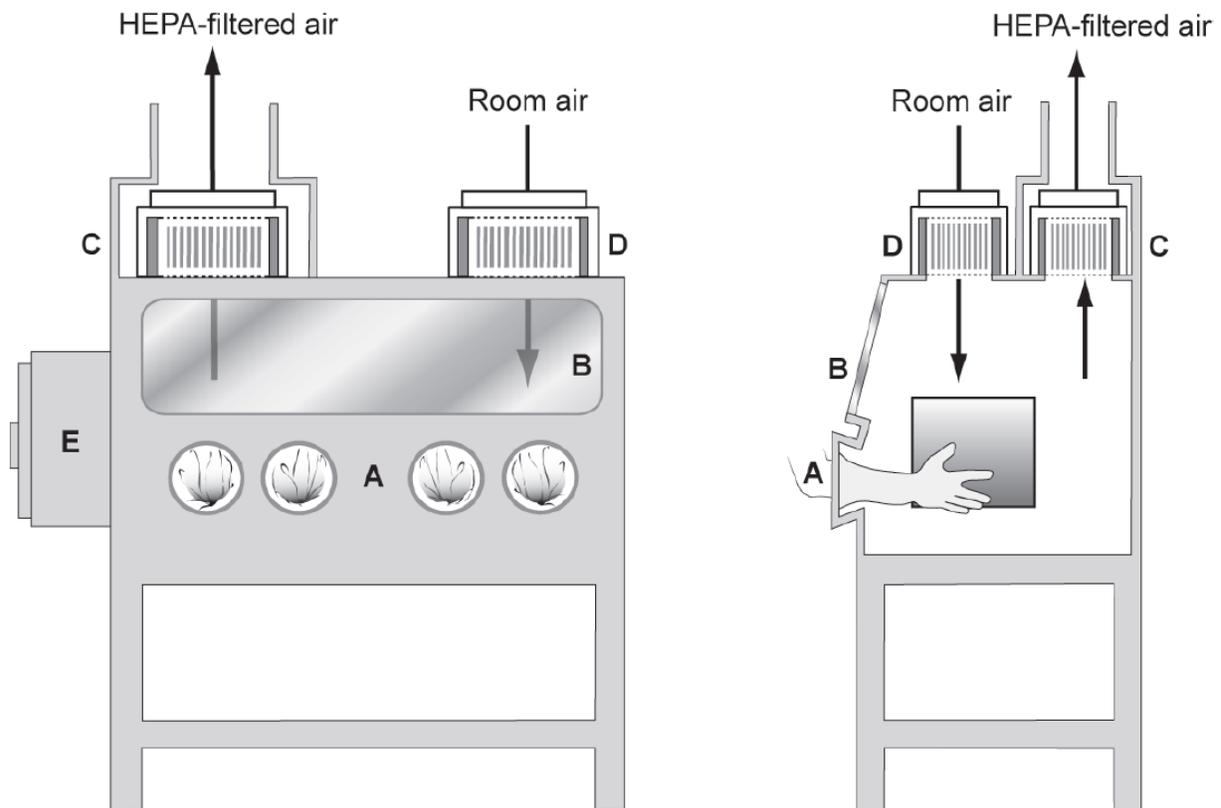
**Figure 16.5** The tabletop model of a Class II, Type A2 BSC (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) positive pressure common plenum; (F) negative pressure plenum. The Class II Type A2 BSC is **not equivalent** to what was formerly called a Class II Type B3 unless it is connected to the laboratory exhaust system. *Note:* The A2 BSC should be canopy connected to the exhaust system.



### C. Class III Cabinets (Figure 16-6)

- The Class III cabinet is totally enclosed, ventilated, and of leak-tight construction.
- Users conduct operations in the cabinet through attached arm-length rubber gloves, which serve as physical barriers.
- The cabinet maintains negative air pressure of at least 0.5 inches water gauge (120Pa).
- The BSC fan draws supply air into the cabinet through HEPA filters.
- Treatment of exhaust air is by double HEPA filtration, or by HEPA filtration and incineration.
- Class III cabinets are used in maximum containment laboratories (BSL-3) and may be used with agents of low, moderate, and high risk.

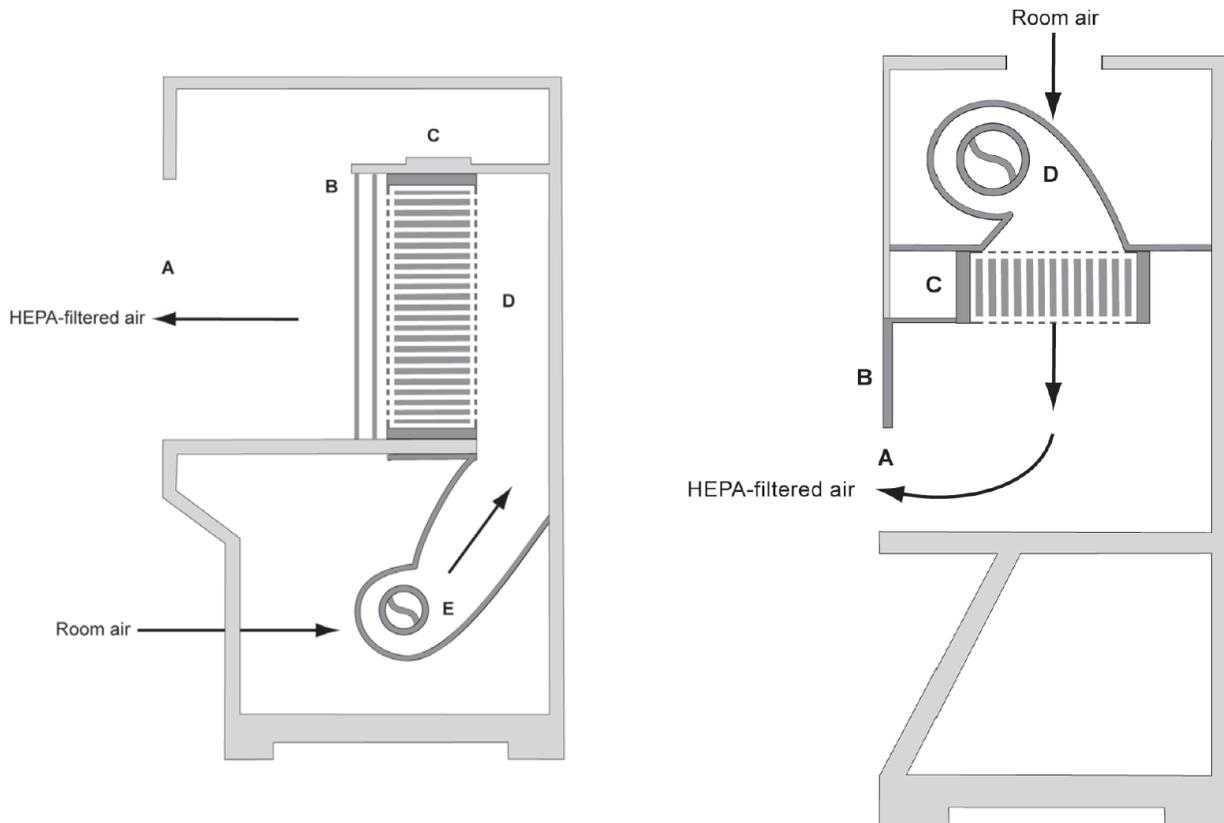
**Figure 16.6** *The Class III BSC* (A) glove ports with O-ring for attaching arm-length gloves to cabinet; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) double-ended autoclave or pass-through box. *Note:* A chemical dunk tank may be installed which would be located beneath the work surface of the BSC with access from above. The cabinet exhaust needs to be hard connected to an exhaust system where the fan is generally separate from the exhaust fans of the facility ventilation system. The exhaust air must be double HEPA-filtered or HEPA-filtered and incinerated.



### III. Laminar Flow Clean Benches (Figure 16-7)

Horizontal and vertical laminar flow clean benches are sometimes mistaken for biological safety cabinets. Clean benches provide product protection but no personnel protection. The horizontal flow clean bench discharges HEPA filtered air across the work surface onto the operator. The less common vertical flow clean bench discharges air downward from a HEPA filter above the work surface. The airflow leaves the work area through the front opening where the operator is located. With both versions of the clean bench, work performed on the work surface could expose the operator to contaminants. Researchers often use clean benches for assembly of sterile apparatus. Do not use clean benches for handling cell cultures, drug formulations, radionuclides, biological hazards, or chemicals. Exposure to some of these materials from manipulation on a clean bench can cause hypersensitivity. Clean benches are to be posted with a label from the [EHS Safety Labels webpage](#) or similar.

**Figure 16.7a** The horizontal laminar flow “clean bench” (A) front opening; (B) supply grille; (C) supply HEPA filter; (D) supply plenum; (E) blower. **Figure 16.7b** The vertical laminar flow “clean bench” (A) front opening; (B) sash; (C) supply HEPA filter; (D) blower. *Note:* Some vertical flow clean benches have recirculated air through front and/or rear perforated grilles.



**Table 16-1. Comparison of Biological Safety Cabinet Characteristics and Applications**

Class, Type	Work Opening	Inflow Velocity (fpm)	Percentage Recirculated Air	Percentage Exhausted Air	Exhaust Volume (cfm) (approximate)	Exhaust Requirement	Application
Class I	Fixed	75	0%	100%	4 ft - 200 6 ft - 300	Exhausted to the outside (remote fan) or to the room through a HEPA filter (integral fan)	Biosafety Level 1-3; small amounts of toxic chemicals or radionuclides (if exhausted to outside)
Class II, type A1	fixed, sliding, or hinged	75-100	70%	30%	4 ft - 300 6 ft - 400	Exhausted to room through HEPA filter or to the environment through an exhaust canopy	Biosafety Level 1-3
Class II, type B1	Sliding	100	30%	70%	4 ft - 250 6 ft - 400	Exhausted to outside, with remote fan; duct is hard connected	Biosafety Level 1-3; small amounts of toxic chemicals or radionuclides
Class II, type B2	sliding, hinged	100	0%	100%	4 ft - 600 6 ft - 1000	Exhausted to outside, with remote fan; duct is hard connected	Biosafety Level 1-3; small amounts of toxic chemicals or radionuclides
Class II, type A2	sliding, hinged	100	70%	30%	4 ft - 300 6 ft - 400	Exhausted to room through HEPA filter or exhausted to outside, with remote fan, utilizing canopy connection	Biosafety Level 1-3; small amounts of toxic chemicals or radionuclides
Class III	Glove ports	N/A	0%	100%	<b>a</b>	Exhausted to outside, through 2 HEPA filters, with remote fan; duct is hard connected	Biosafety Level 1-4; small amounts of toxic chemicals or radionuclides

**a** Class III cabinets should have approximately 20 air changes per hour or enough ventilation to accommodate the heat load. The cabinet must maintain a negative pressure of 0.5 in. w.g. and should maintain 100 fpm through a glove port, if a glove is accidentally removed.

## **V. Selection of Biological Safety Cabinets**

The selection of the class or type of BSC depends on the degree or nature of the hazard associated with the experiment, the aerosol-producing potential of the laboratory procedures, and the requirement to protect the experiment from airborne contamination. EHS must review and approve all purchases and installations of biological safety cabinets. This ensures selection of the proper cabinet for the intended use, and installation for proper functioning, maintenance, and certification.

Table 16-1 provides a summary and comparison of BSC characteristics and application. In general, the Class II, type A1, A2 BSC (non-ducted) is sufficient when the work involves infectious agents and cell culture work in the absence of volatile toxic chemicals and radionuclides. When small amounts of volatile chemicals or radionuclides are involved, the Class II, type B (types B1, B2) is more appropriate. Select a Class III cabinet when the work involves the handling of high-risk microbiological agents, concentrated amounts of carcinogens, or highly toxic chemicals.

NSF International - The Public Health and Safety Company™ lists the models of the various cabinet manufacturers that meet its standard criteria described in NSF Standard No. 49 for “Class II (laminar flow) Biohazard Cabinetry”. This standard includes design and construction requirements as well as performance specifications such as vibration; temperature rise; noise level, and personnel, product, and cross-contamination protection determined by a spore aerosol challenge. Selection of an NSF-approved model assures the purchaser that the model meets minimum construction and performance standards.

Most manufacturers offer user options such as ultraviolet (UV) or germicidal lights. UV lights are not recommended by NSF 49, but if installed they must be cleaned weekly to remove dust which can reduce their germicidal effectiveness. They must also be installed in a manner that does not reduce the required performance of the cabinet. . Take precautions to prevent exposure of the eyes and skin to damaging effects of UV light. Keep the protective sashes in place, and do not place your hands or arms within a cabinet when the UV (purple) lamp is on. Turn off UV lamps when the room is occupied.

To accommodate special applications, the manufacturer can modify the design of biological safety cabinets. Examples include altering the front sash to accommodate microscope eyepieces, or adapting the work surface to include a centrifuge or animal waste handling capabilities.

## **VI. Installation and Certification of Biological Safety Cabinets**

### **A. Installation**

The cabinet should be located in a space that is free from drafts and traffic. Air conditioning vents, opening doors, and personnel traffic can produce air currents, which may penetrate the air barriers at the front opening of the cabinet. The ideal location for BSCs is a “dead-end” area of the laboratory. Nearby HVAC vents should be directed away from the BSC. Provide a

clearance of 12-14 inches (30-35 cm) above Class II, type A1, A2, cabinets to allow access for accurate exhaust flow measurements and filter replacement.

The Class II, type A1 or A2 BSC is built to exhaust air back into the laboratory; therefore, it is generally best not to connect a duct to the cabinet. If a duct is connected by a canopy, the remote exhaust fan must be the correct size to match precisely the exhaust air volume from the cabinet. The canopy duct must not interfere with requirements for certification tests and filter replacement. Make sure the exhaust filter is accessible.

Type A or A2 cabinets with a canopy connected and exhausted by a remote fan should have an audible and visual alarm to indicate a loss of exhaust airflow.

The exhaust fan should interlock with the cabinet fan switch, so that both fans are either “on” or “off” at the same time. If the cabinet fan is “off” on a Class II, type A1 or A2 cabinet but the exhaust fan is “on”, the exhaust fan will pull room air contaminants through the cabinet and the supply filter in the opposite direction of normal cabinet operation. The clean side of the supply filter is therefore contaminated. When someone turns “on” the cabinet, the airflow through the supply filter is now flowing in the correct direction, and contaminants can dislodge from the filter media into the clean work area of the cabinet.

Class II, type B1 and B2 cabinets by design must duct to the outside using a remote exhaust fan, usually located on the roof. Once the cabinet is set or certified in its acceptable airflow range, audible and visual alarms shall be required to indicate a 20% loss of exhaust volume within 15 seconds. The internal cabinet fans shall be interlocked to shut off at the same time the alarms are activated.

To size the exhaust fan correctly, consult the cabinet manufacturer to obtain the pressure drop through the cabinet with fully loaded filters. For some older models, the exhaust filter is located on the roof just upstream from the fan. The maintenance technician uses a bag-in / bag-out filter assembly so that he/she does not have to handle a contaminated filter directly. Upstream and downstream certification test ports and isolation dampers for formaldehyde gas decontamination are also necessary.

The use of open flame burners is not allowed in Biological Safety Cabinets of Type A1 and Type A2 at UNC. Biosafety Cabinets of Type A1 and Type A2 currently in use must have their gas lines removed or disconnected. New BSC installations of Type A1 and Type A2 will not be connected to gas lines. The full [Policy on the use of Flammable Gases in Biological Safety Cabinets](#) is on the [UNC website](#).

## **B. Certification**

A BSC certifier tests and evaluates the performance of each BSC after initial installation in the laboratory, prior to use, whenever moved, and periodically thereafter. The following are typical field tests: downflow velocity profile for the supply air, work access opening airflow, HEPA filter leak test, cabinet integrity test, and airflow smoke patterns.

NSF Standard No. 49 describes certification field tests. NSF also accredits BSC certifiers. Recertification of the cabinet is necessary when the HEPA filters are changed, maintenance repairs are required, or when a cabinet is relocated. BSCs require decontamination with formaldehyde gas before maintenance work or filter changes, after gross spills of biohazardous materials, and before moving the cabinet.

Contact EHS for recommended certifiers at UNC.

## **VII. Procedures for the Proper Use of a Class II Biological Safety Cabinet**

Any laminar flow BSC is only a supplement to good microbiological techniques, not a replacement. If users do not properly understand or operate cabinets, they will not maintain an adequate protective barrier between the operator and the experiment. Listed below are procedures for proper use of BSCs.

- Turn the cabinet fan and the fluorescent light on. Turn off the UV light. Confirm that the drain valve is closed.
- Wipe the work surface with 70% ethanol or other appropriate disinfectant. Let the unit run for 5 -10 minutes to clean itself before beginning work.
- Plan the work operation in advance. Place everything needed for the complete procedure in the cabinet before starting. Nothing should pass through the air barrier, either in or out, until the procedure is complete. Arrange materials in a logical manner such that clean and contaminated materials are segregated. Remove from the cabinet all materials or equipment not necessary for the particular procedure.
- Avoid placing materials on the air intake grille, at the front of cabinet as this disrupts the protective air barrier.
- Keep equipment at least four inches (10 cm) inside the cabinet work area. Perform manipulations of hazardous materials as far back in the work area as possible.
- After the procedure is completed, decontaminate all equipment in direct contact with the research agent with an appropriate disinfectant. Run the cabinet at least three minutes with no activity to purge airborne contaminants from the work area.
- After removal of all materials and equipment, wipe the work surface with 70% ethanol or other appropriate disinfectant. Clean any spilled culture media that may support fungal growth and cause contamination in subsequent experiments.
- Turn off the cabinet fan. Some researchers prefer to let the cabinet run continuously. You may turn on the UV light if the BSC is so equipped and there are barriers in place to prevent inadvertent UV exposure to other lab personnel.