

BIOSAFETY CABINETS

Biosafety Cabinet Classification

Biological Safety Cabinets (BSCs) are designed to provide personnel, product and/or 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 research and clinical lab.

When Class I and II BSCs are properly maintained and used in conjunction with good microbiological techniques, they provide an effective containment system for safe manipulation of moderate and high-risk microorganisms (Biosafety Level 2 and 3 agents). 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 protection for the research material itself through high-efficiency particulate air filtration (HEPA filtration) of the airflow down across the work surface (vertical laminar flow). Class III cabinets offer the maximum protection to laboratory personnel, the community, and the environment because all hazardous materials are contained in a totally enclosed, ventilated cabinet.

All classes of BSCs are equipped with exhaust HEPA filters which distinguishes them from “clean benches.” Class II and III BSCs and “clean benches” protect the products/cultures within from environmental contamination by using a supply air HEPA filter. Class I BSCs are not equipped with supply HEPA filters. A “clean bench,” although providing 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. Exhaust design and other factors are used to classify BSCs, as indicated in Table 1.

Further information can be found in Appendix A of the [CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Ed. \(2009\)](#)

The [National Sanitation Foundation \(NSF\) Biosafety Cabinetry Program](#) developed NSF/ANSI Standard 49 for the evaluation of Class II laminar flow biological safety cabinets. Information regarding the certification process and NSF-accredited certifiers can be found at their site.

Table 1. Characteristics and 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)	Any application where the product is not hazardous, but must be kept contaminant free. A laminar flow clean bench provides HEPA filtered supply to the work surface and a particulate-free work area. Examples of processes that are appropriately performed in this type of cabinet include: preparation of nonhazardous intravenous mixtures and media; particulate-free assembly of sterile equipment and electronic devices; polymerase chain reaction (PCR). (Figures 8, 9)	Product only	Not Acceptable
BSC Class I	Inward (≥ 75)	In at front, exhaust through HEPA to the outside or into the room through HEPA (Figure 1)	Personnel and Environment	Acceptable if hard ducted
BSC Class II, Type A1	Inward (75)	Laminar flow device; 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 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 (pre- 2002 was A/B3)	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)	Laminar flow device; 30-40% of airflow is recirculated and the 60-70% balance 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)	Laminar flow device with dedicated HEPA filtered supply to the work surface and total exhaust to the outside through a HEPA filter and the building exhaust system. No recirculation, exhaust must be hard ducted to the outside. (Figure 6)	Product, Personnel, and Environment	Acceptable
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. (e.g., pharmaceutical manufacturing of potent compounds, BSL-4 agents). (Figure 7)	Maximum Product, Personnel, and Environment	Limited amounts*

***In no circumstances should the chemical concentration approach the lower explosion limits of the compound. 2:1**

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

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

Figure 1 Class I Biological Safety Cabinet

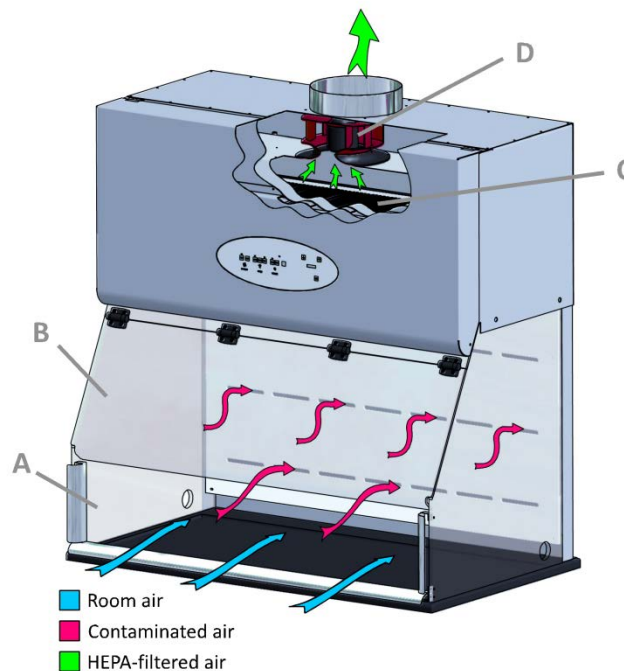


Photo courtesy [NuAire, Inc. Laboratory Equipment Supply](#)

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.

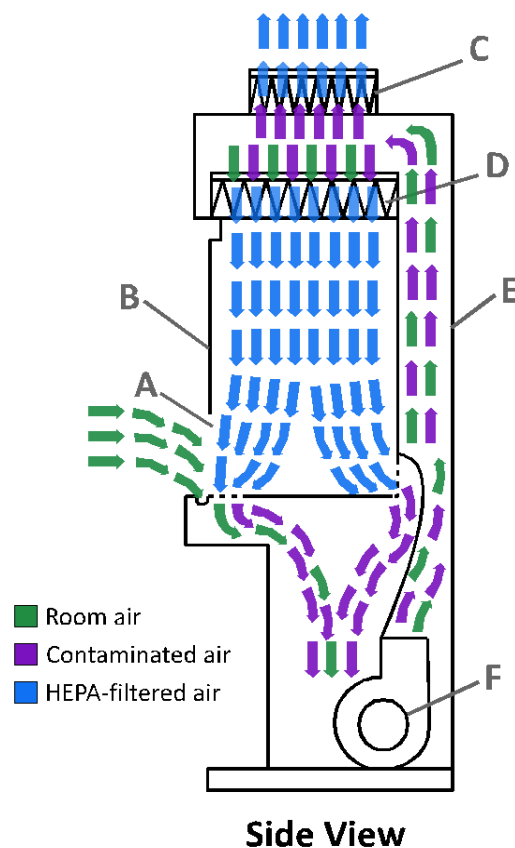
The classical Class I BSC is hard-ducted (i.e., direct connection) to the building exhaust system, and the building exhaust fan provides the negative pressure necessary to draw room air into the cabinet.

Class II BSC

Class II BSCs are partial barrier systems that rely on the 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) the potential for contaminant release into the laboratory work environment is increased as is the risk of product contamination. Class II (Types A1, A2, B1 and B2)¹ BSCs provide personnel, environmental 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 certified 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.

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.

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 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).

Figure 3
Class II, Type A2

Air In-flow 70% Recirculated, 30% Exhausted

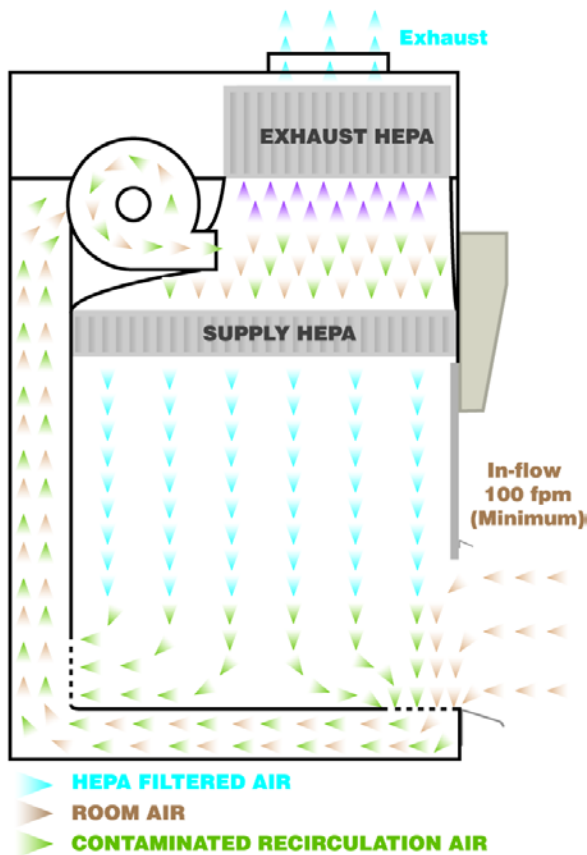
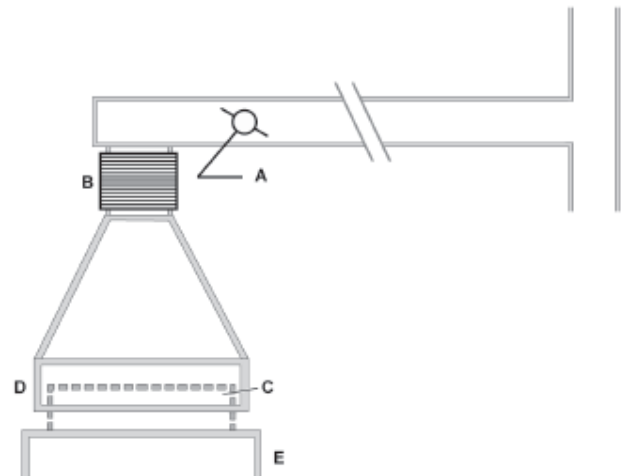


Photo courtesy
[NuAire, Inc. Laboratory Equipment Supply](http://www.nuaire.com)

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 and 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 downflow 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

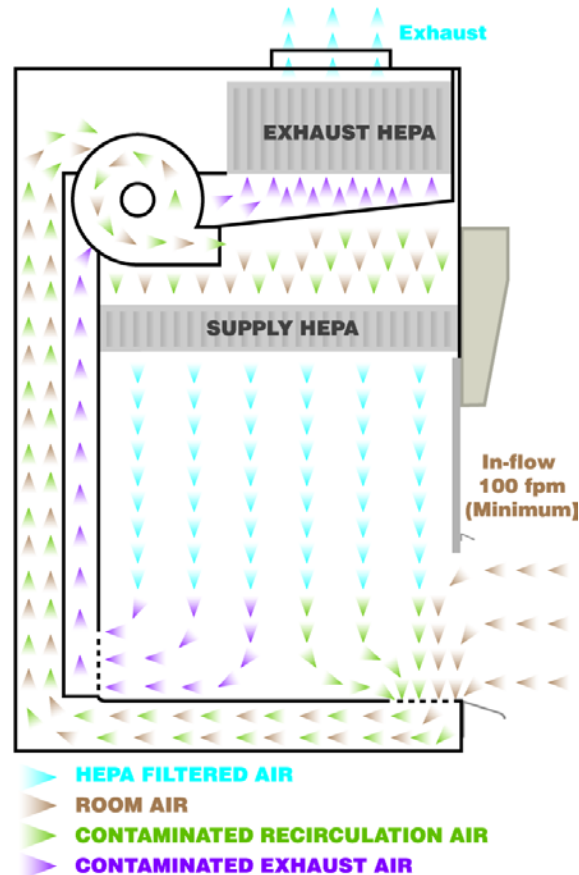


Photo courtesy
[NuAire, Inc. Laboratory Equipment Supply](http://www.nuaire.com)

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

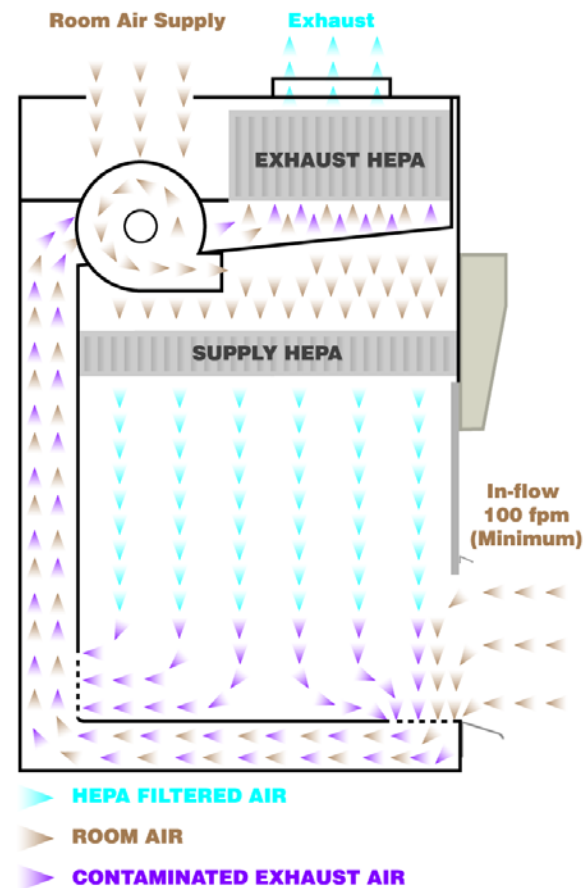


Photo courtesy
[NuAire, Inc. Laboratory Equipment Supply](http://www.nuaire.com)

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

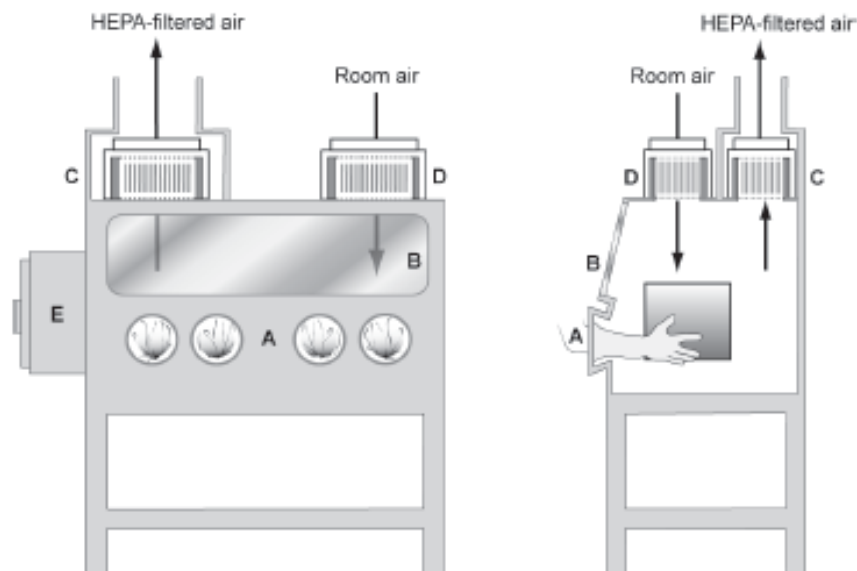
Class II, Type B2, BSCs (Figure 6) are total exhaust cabinets; no air is recirculated. This cabinet provides simultaneous primary biological and chemical containment. Consideration must be given to the chemicals used in BSCs as some chemicals can destroy the filter medium, housings, and/or gaskets causing 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, capturing the supply air plus the additional amount of room air needed to produce a minimum calculated or measured inflow face velocity of 100 lfpm. All air entering this cabinet is exhausted, and passes through a HEPA filter (and perhaps some other air-cleaning device such as a carbon filter if required for the work being performed) prior to discharge to the outside.

Class III BSC

The Class III BSC (Figure 7) 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 non-opening 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 III Biological Safety Cabinet²



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 only provide product 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 8 Horizontal laminar flow “clean bench”

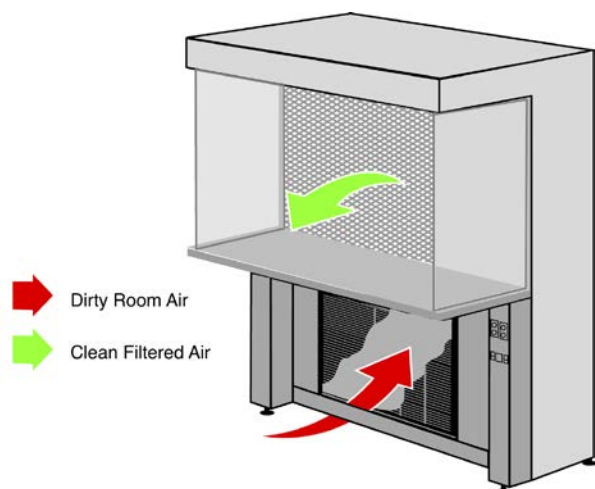


Photo courtesy
[NuAire, Inc. Laboratory Equipment Supply](http://www.nuaire.com)

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

Figure 9 Vertical laminar flow “clean bench”

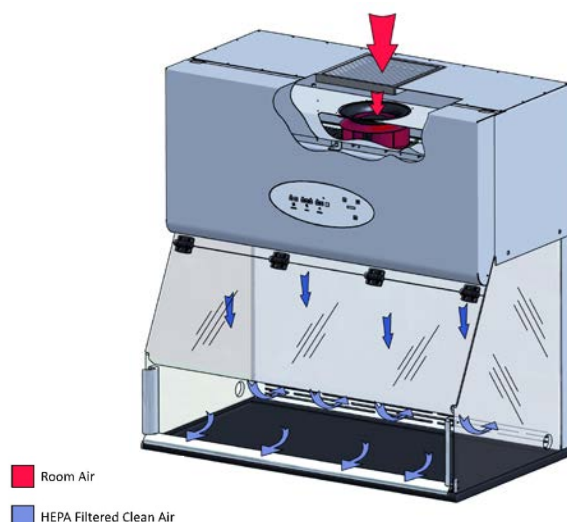


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[NuAire, Inc. Laboratory Equipment Supply](http://www.nuaire.com)

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

Chemical and Radioisotope Use in BSCs

In general, BSCs are not designed for use with volatile chemicals or radionuclides. HEPA filters will not “trap” vapors thereby exposing laboratory workers and others in the building when the vapors are discharged back into the laboratory and picked up by the general building ventilation system.

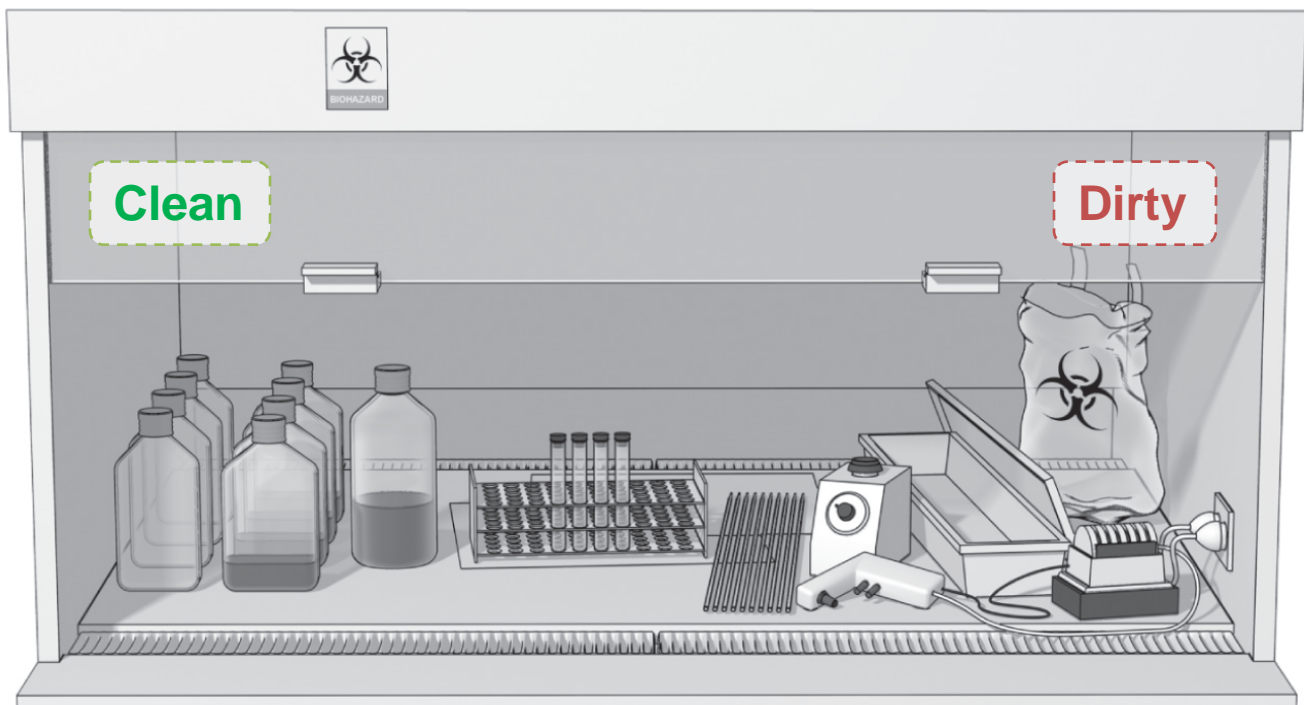
- HEPA filters, gaskets, and their housing assemblies can also be damaged by some chemicals thereby compromising worker and environmental protection.
- BSCs are not designed with intrinsically-safe electrical components (i.e., switches, lights, etc.). If sufficient concentrations of flammable vapors are present in the cabinet (e.g., at or above the Lower Explosive Limit, LEL), a fire or explosion may result.
- The low face velocity of Class I and Class II, Type A1 BSCs is insufficient to contain chemical vapors.
- Relatively small amounts of non-volatile chemicals and radionuclides can be used in all BSCs, but amounts should be restricted to minute quantities when using a Class II, Type A1 BSC (due to the low face velocity and design of discharge air).
- Small quantities of volatile chemicals can be used in a Class II, Type B2 and Class III BSCs. The amount in process should never be in a quantity that can generate vapor concentrations at or near the LEL.

Using Biological Safety Cabinets

- Read the operator’s manual and follow all manufacturer’s recommendations.
- When designing the laboratory, locate the cabinet in an area where it will not be adversely affected by air currents:
 - Away from pedestrian traffic and doors.
 - Away from other room ventilation devices (e.g., supply diffusers, fans, fume hoods, etc.).
- Stool height should be adjusted so that the investigator’s face is above the front opening.
- Restrict use of the cabinet to one person at a time. Exceptions to this would be for certain procedures requiring two people or limited to experienced and highly trained personnel and only when working in 6’ or 8’ biosafety cabinets.
- If the cabinet is off before you begin work, turn it on and allow it to run for at least 5 minutes while you are preparing your supplies.
- Wipe the surfaces of items placed in the cabinet for a work session with 70% ethanol or isopropanol. This will reduce the introduction of contaminants to the cabinet environment.
- Materials or equipment placed inside the cabinet may cause disruption of the airflow, resulting in turbulence, possible cross-contamination and/or breach of containment. Extra supplies (e.g., extra gloves, culture plates or flasks, culture media) should be stored outside the cabinet.
- Movement of arms into and out of the cabinet can disrupt airflow and affect cabinet performance. It is advisable to use a checklist to ensure that all necessary items for a specific activity have been placed in the cabinet prior to starting a procedure. Only materials needed for the particular procedure should be in the hood since many items in a hood can cause turbulence and disruption of the airflow.

- If it is necessary to move arms in and out of the cabinet, this should be done slowly and with movement perpendicular to the face of the cabinet. Do not block the front grille by resting arms on them or by putting notes or any other materials on them.
- Manipulation of materials should be delayed for one minute after putting arms in hood to allow the hood environment to stabilize.
- Work at least 4" to 6" from the opening of the cabinet. Work as far back in the cabinet as is feasible since exhaust air is primarily drawn from the back of the cabinet.
- Do not use a Bunsen burner in a biosafety cabinet. The flame can produce eddy currents, which can push infectious agents out of the cabinet towards the worker. In addition, build-up of flammable gases can result in a fire or explosion. Electric incinerators (Bacti-cinerator®) are recommended for sterilization of loops. Another alternative to a standard Bunsen burner is a Touch-o-Matic® Bunsen burner which is activated only when resting your wrist on a trigger; there is not a constant flow of gas like with a regular Bunsen burner.
- Personal protective clothing such as a laboratory coat and gloves must be worn when working at the hood. A solid front, back closing gown provides the best protection. Gloves should be pulled over the knitted wrists of the gown or lab coat. If two pairs of gloves are worn, put the first pair under and the second pair over the wrist of the gown or lab coat. Elasticized sleeves can also be worn to protect the investigator's wrists.
- Carry out work on an absorbent pad to contain small spills. Ensure that the pad does not cover the front grille opening.

Figure 10 A typical lay out for working “clean to dirty” within a Class II BSC.2



Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right). This arrangement is reversed for left-handed persons.

- Clean up spills as soon as they occur. Remove and disinfect the grill if contaminated.
- When working in a Class III cabinet, ensure that contaminated items or spill clean-up components are immediately removed to the decontamination module or dunk tank as soon as possible.
- Work from “clean” to “dirty” areas. Place contaminated materials toward the rear of the cabinet. (Figure 10)
- All contaminated items used in the biosafety cabinet should be placed into a biohazard bag, discard tray or other suitable container prior to removal from the biosafety cabinet. Additionally, these containers should be surface decontaminated prior to removal from the biosafety cabinet.
- For biohazard bags closed in the cabinet prior to removal from the cabinet, remember to add a small amount of water to the bag to generate steam in the autoclave.
- Place used pipettes in horizontal trays containing appropriate disinfectant. Dropping pipettes into vertical receptacles creates aerosols. Removing contaminated pipettes from the cabinet to place in a biohazard bag or vertical waste receptacle is unnecessary and may result in contaminating the area outside the cabinet.
- Disinfect the cabinet surfaces after use with a disinfectant that is appropriate for the agent in use. Be sure to allow sufficient contact time for the disinfectant.
- Never attempt to remove or change the HEPA filters.
- Leave the fan blower on in the cabinet for a short period of time (5 to 10 minutes) after finishing work to allow the system to purge. Some safety cabinets should be left on at all times. Check with your supervisor for operation procedures.
- Biosafety cabinets must be decontaminated before being moved or relocated. Please refer to the ***EHS SOP Decontamination of Laboratory Equipment*** for advice on the necessary method of decontamination. If pathogens or human cell lines were used in the cabinet, gas decontamination is required in addition to surface decontamination of the cabinet.
- Ensure that the cabinet is certified upon installation, modification, movement, and annually.

Use of UV Lights in Biological Safety Cabinets³

The Centers for Disease Control (CDC) and the National Institute of Health (NIH) agree that UV lamps are **not recommended, nor required** in biological safety cabinets. The activity of UV lights for sterilization/decontamination purposes is limited by a number of factors, including⁷:

- **Penetration** - In a dynamic air stream (e.g., biological safety cabinet), UV light is not penetrating. Microorganisms beneath dust particles or beneath the work surface are not affected by the UV irradiation. UV irradiation can cause erythema that may damage both the skin and eyes of laboratory employees. The surface and outer tissue layers of eyes and skin are primarily involved because UV does not penetrate deeply into tissue. These effects are generally not permanent but can be quite painful.
- **Relative Humidity** - Humidity adversely affects the effectiveness of UV. Above 70% relative humidity, the germicidal effects drop off precipitously.
- **Temperature and Air Movement** - Optimum temperature for output is 77°-80°F. Temperatures below this optimum temperature result in reduced output of the

germicidal wavelength. Moving air tends to cool the lamp below its optimum operating temperature and therefore results in reduced output.

- **Cleanliness** - UV lights should be cleaned weekly with an alcohol and water mixture as dust and dirt can block the germicidal effectiveness of the ultraviolet lights.
- **Age** – UV lamps should be checked periodically (approximately every six months) to ensure the appropriate intensity of UV light is being emitted for germicidal activity (UV C). The amount of germicidal wavelength light emitted from these bulbs decreases with age and bulb ratings (hours of use) may vary by manufacturer.

The information provided in this SOP supplements the UNL Bloodborne Pathogen Exposure Control Plan and UNL Biosafety Guidelines. Please refer to the full program documents for more information.

¹ NSF International (NSF); American National Standards Institute (ANSI). (2009, April 20). Class II (laminar flow) biosafety cabinetry. NSF/ANSI Standard 49-2009. 10th, 148. Ann Arbor, MI: NSF International.

²Centers for Disease Control and Prevention, N. I. (2007). Biosafety in Microbiological and Biomedical Laboratories (5th Edition ed.). Washington, DC: U.S. Government Printing Office.

³Burgener, J. (2006). Position Paper on the Use of Ultraviolet Lights in Biological Safety Cabinets. Applied Biosafety , 11 (4), 228-230.