

# Personnel & Product Protection: A Guide To Biosafety Enclosures



An Industry Service Publication

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## Foreword

*This booklet was developed as a guide to selecting a biosafety enclosure that provides personnel and/or product protection. The information presented is unbiased and generic in nature compiled with help from experienced microbiologists, engineers and safety enclosure users. While this booklet should raise the questions necessary to identify your specific enclosure requirements, it may not answer those questions. Only you and your safety officer or industrial hygienist can identify your laboratory's unique requirements.*

## Types of Laboratory Enclosures

Different types of enclosures provide protection and containment in the laboratory. Before selecting an enclosure for your particular application, a thorough review of the work and a risk assessment should be performed. Briefly, the major types of enclosures include:



◀ Labconco Protector®  
Premier® Laboratory Hood

### **Laboratory Fume Hoods.**

Laboratory fume hoods are ventilated enclosures that capture, contain, and remove chemical fumes and vapors from the laboratory.

The fume hood provides protection by

an inward flow of room air through the work area of the hood and out of the laboratory through an exhaust system. Fume hoods are designed to control chemical fumes and vapors that are hazardous in high concentrations, and should not be used with materials that are hazardous in lower concentrations. Laboratory fume hoods should never be used to contain biohazardous materials, as the contaminated exhaust is released directly to the outside environment. Finally, because room air is drawn through the work area, fume hoods do not protect the materials inside from contaminants in the air. If your application requires a chemical fume hood, Labconco's industry service publication, *How To Select The Right Laboratory Hood System*, may provide helpful reading. To request your free copy, call Labconco at 800-821-5525 or 816-333-8811.

**Clean Benches.** Clean benches use a blower to force air through a High Efficiency Particulate Air (HEPA) filter, and over a work surface. The filtered air may be directed vertically or horizontally across the work area, as shown in Figure 1. This laminar flow of clean air protects materials in the work area from particulate and cross contamination. Clean benches were developed as part of "clean room" technology, and are widely used in the electronics and pharmaceutical industries. They have been successfully used in research laboratories for tissue culture and media preparation. The major limitation of the clean bench is it only provides product protection; the operator is constantly exposed to any aerosols generated in the work area. Thus, work with toxic, allergenic or biohazardous materials should not be performed in a clean bench.



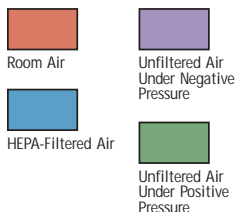
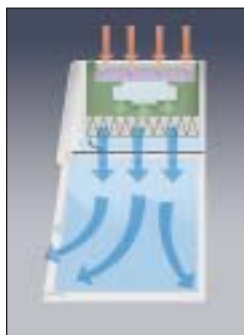
◀ Labconco Purifier®  
Horizontal Clean Bench



Labconco Protector® Multi-Hazard Glove Box is a type of ventilated glove box.



Figure 1.  
Horizontal Clean Bench (left) and  
Vertical Clean Bench (below)



**Controlled Atmosphere Glove Boxes.** Controlled atmosphere glove boxes, sometimes called dry boxes, are enclosures that maintain a leak-free environment so that experiments may be carried out under controlled conditions, as in the absence of oxygen or moisture, in an atmosphere of inert gas or under controlled pressure. Controlled atmosphere glove boxes are widely used for oxygen-sensitive, inorganic, organic, organometallic, and non-hazardous biochemical materials. The controlled atmosphere glove box



Labconco Protector® Controlled Atmosphere Glove Box

should not be used with biohazardous materials because it has no means to capture aerosols generated within the work area.

**Ventilated Glove Boxes** fitted with appropriate filters are enclosures that protect the product inside the work area from particulate contamination, and protect personnel from potentially hazardous materials by filtration and a physical barrier. Applications for ventilated glove boxes include weighing reactive solids, loading capillary tubes for x-ray diffraction, and transfer of low level radioactive, carcinogenic and toxic materials. The Class III Biosafety Cabinet is a type of ventilated glove box, specially designed to sterilize or disinfect materials before they enter or exit the work area of the enclosure. Other ventilated glove boxes should not be used with biohazardous materials, as there is no way to disinfect materials before they are removed from the work area.



Labconco Purifier® Delta® Series Class II Biological Safety Cabinet

Biological Safety Cabinets

The terms biological safety cabinet and biosafety cabinet have been widely used to describe a variety of containment devices equipped with HEPA filter(s), designed to provide personnel or both personnel and product protection from biohazardous materials. The terms should only be applied to those devices that meet the requirements of Class I, II, or III specifications, based on their construction, airflow velocities and patterns, and their exhaust systems.

Biohazards and Biosafety Levels

The word biohazard is a contraction of the words biological and hazard, and defined as: "an infectious agent, or part thereof, presenting a real or potential risk to the well-being of man, animals and/or plants, directly through infection or indirectly through disruption of the environment."\*

Biosafety Levels 1 through 4 were established by the Centers for Disease Control (CDC) and the National

Biosafety Level 1

Practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. *Bacillus subtilis*, *Naegleria gruberi*, infectious canine hepatitis virus, and exempt organisms under the NIH Recombinant DNA Guidelines are representative of those microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Vaccine strains that have undergone multiple in vivo passages should not be considered avirulent simply because they are vaccine strains.

Biosafety Level 1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing.

Biosafety Level 2

Practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, HIV, the salmonellae, and *Toxoplasma* spp. are representative of microorganisms assigned to this containment level. Biosafety Level 2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the OSHA Bloodborne Pathogen Standard for specific, required precautions.)

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at Biosafety Level 2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a Biological Safety Cabinet or safety centrifuge cups. Other primary barriers should be used as appropriate, such as splash shields, face protection, gowns, and gloves. Secondary barriers such as handwashing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

Biosafety Level 3

Practices, safety, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

At Biosafety Level 3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a Biological Safety Cabinet or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

Biosafety Level 4

Practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigen relationship to Biosafety Level 4 agents also should be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at Biosafety Level 4.

The primary hazards to personnel working with Biosafety Level 4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals pose a high risk of exposure and infection to laboratory personnel, the community and the environment.

The laboratory worker's complete isolation of aerosolized infectious materials is accomplished primarily by working in a Class III Biological Safety Cabinet or a full-body, air-supplied positive-pressure personnel suit. The Biosafety Level 4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment.

From *Biosafety in Microbiology and Biomedical Laboratories*, U.S. Department of Health and Human Services, HHS publication (CDC) 99-8395, 4th ed. April 1999. Available for downloading from CDC's website at [www.cdc.gov](http://www.cdc.gov).

Table 1. Biosafety Levels

Biosafety Level	1	2	3	4
Infectious Agents	Not known to consistently cause disease in healthy adults.	Associated with human disease. Primary hazards are percutaneous injury, ingestion, mucous membrane exposure.	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences.	Dangerous/exotic agents that pose a high risk of life-threatening disease. Aerosol-transmitted lab infections or related agents with unknown risk of transmission.
Examples	<i>Bacillus subtilis</i> <i>Naegleria gruberi</i> Infectious canine hepatitis virus <i>E. coli</i>	Measles virus Salmonellae <i>Toxoplasma</i> spp. Hepatitis B virus	<i>M. tuberculosis</i> St. Louis encephalitis virus <i>Coxiella burnetii</i> <i>Bacillus anthracis</i> (production levels)	Ebola Zaire Sin Nombre virus Rift Valley Fever
Practices and Techniques	Standard microbiological practices	Level 1 plus: <ul style="list-style-type: none"> <li>Limited access</li> <li>Biohazard warning signs</li> <li>"Sharps" precautions</li> <li>Biosafety manual defining any needed waste decontamination or medical surveillance policies</li> <li>Respiratory protection as required</li> </ul>	Level 2 plus: <ul style="list-style-type: none"> <li>Controlled access</li> <li>Decontamination of waste</li> <li>Decontamination of lab clothing</li> <li>Baseline serum samples of lab personnel</li> </ul>	Level 3 plus: <ul style="list-style-type: none"> <li>Clothing change before entry into lab</li> <li>Shower on exit</li> <li>All material decontaminated on exit from lab</li> </ul>
Safety Equipment (Primary Barriers)	None required	Primary barriers: Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials. Personal protective equipment: lab coats, gloves, face protection as needed	Primary barriers: Class I or II BSCs or other physical containment devices used for all open manipulations of agents. Personal protective equipment: lab clothing, gloves, respiratory protection as needed	Primary barriers: <ul style="list-style-type: none"> <li>All procedures conducted in Class III or I or II BSCs in combination with full-body, air-supplied, positive-pressure personnel suit</li> </ul>
Facilities (Secondary Barriers)	<ul style="list-style-type: none"> <li>Open benchtop sink required</li> </ul>	Level 1 plus: <ul style="list-style-type: none"> <li>Autoclave available</li> </ul>	Level 2 plus: <ul style="list-style-type: none"> <li>Physical separation from access corridors</li> <li>Self-closing, double door access</li> <li>Exhaust air not recirculated</li> <li>Negative airflow into laboratory</li> </ul>	Level 3 plus: <ul style="list-style-type: none"> <li>Separate building or isolated zone</li> <li>Dedicated supply/exhaust vacuum, and decontamination systems</li> <li>Additional requirements as outlined in <i>Biosafety in Microbiological and Biomedical Laboratories</i></li> </ul>

Table 2. Summary of Biosafety Levels and Infectious Agents

Institutes of Health (NIH) and are combinations of laboratory practices and techniques, safety equipment and facilities. All of these levels are appropriate for the biohazard posed by the agents used and for the laboratory activity.

\*NSF International Standard/American National Standard for Biosafety Cabinetry—Class II (Laminar Flow) Biosafety Cabinetry, Ann Arbor, Michigan

The complete definitions and summary of Biosafety Levels 1 through 4 are described in Tables 1 and 2. For further information regarding Biosafety Levels, refer to *Biosafety in Microbiology and Biomedical Laboratories*, U.S. Department of Health and Human Services, HHS publication (CDC) 99-8395, 4th edition, April 1999.

## History of the Biological Safety Cabinet

The biosafety cabinet began as a microbiological isolation box developed in 1909. Prefiltered air was drawn through the cabinet, maintained under negative pressure, and exhausted through a flask containing disinfectant. Van den Ende developed the first "modern" biological safety cabinet in 1943 at the National Institute for Medical Research in London, England. A gas burner in the exhaust stack created an inward movement of air into the enclosure and incinerated the exhaust. The design was refined, and by 1953, recognizable predecessors of the modern Class I cabinet were in use, albeit with spun glass exhaust filters.

Glass wool or cotton filters are, at best, only 95% efficient, so exhaust air frequently had to be incinerated as well, and recirculation of the exhaust air was unthinkable. In 1962 the HEPA filter was developed, which was 99.5 to 99.99% efficient. This allowed for filtered air to be exhausted directly outside the laboratory, or to be recirculated in the cabinet, leading to the establishment of the current Class I, Class II, and Class III cabinets available today.

## HEPA Filters

The HEPA filter is the heart of the biosafety cabinet. The HEPA filter is a disposable dry-type filter, constructed of boron silicate microfibers cast into a thin sheet, much like a piece of paper. Although the media is a flat sheet, the glass microfibers form a complex three dimensional matrix that traps particulate matter. The filter media is folded, to increase its surface area, and corrugated aluminum separators or lines of glue may be placed between the layers to allow the air to penetrate to the deepest part of the pleat (Figure 2).

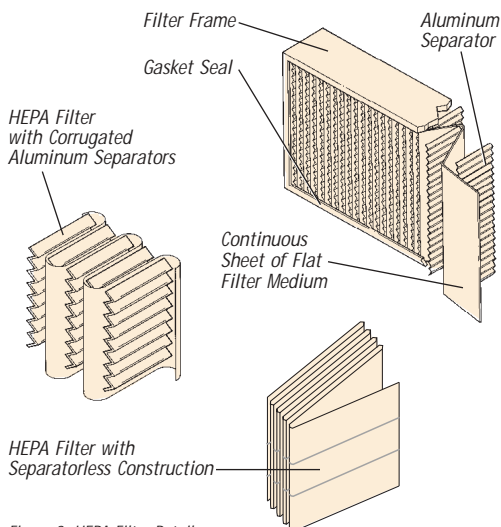


Figure 2. HEPA Filter Details

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The HEPA filter is a particulate filter, retaining airborne particles and microorganisms, however, gases pass freely through the filter. HEPA filters retain particulate matter by five distinct mechanisms: sedimentation, electrostatic attraction, interception, inertial impaction, and diffusion.

Sedimentation occurs when a particle settles onto a filter fiber due to gravitational force. Electrostatic attraction is the attraction of a particle to the filter fiber due to its opposite electrical charge. These are the least effective mechanisms of particulate removal by HEPA filtration.

Interception is dependent on particle size and occurs when a particle follows the air stream onto a filter

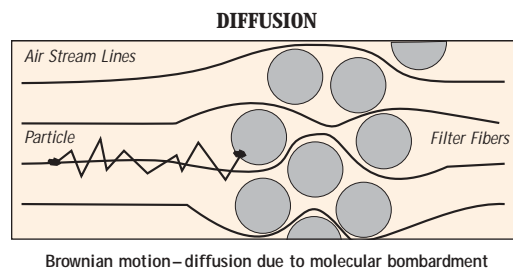
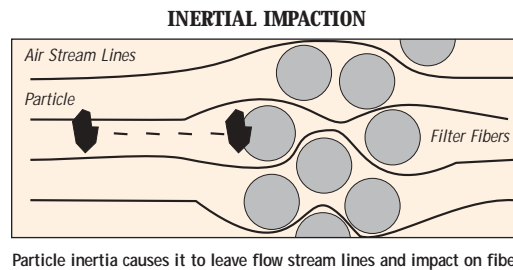
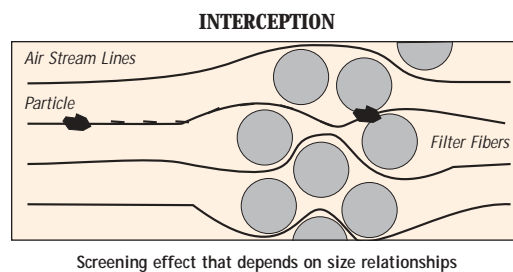


Figure 3. Air Filtration Theory Particle Collection Mechanisms

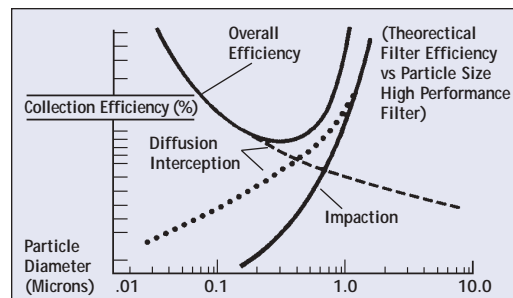


Figure 4. Relative Effect of Particle Collection Mechanism



fiber, and is retained. Inertial impaction occurs when a large particle leaves the air stream to be impacted directly on the filter fiber. Diffusion occurs with very small particles, and is aided by the Brownian (random) motion of the particle (Figures 3 and 4).

HEPA filters are rated on their ability to retain particles 0.3 micron ( $\mu\text{m}$ ) in diameter. The filters are most commonly tested by injecting an aerosol of Dioctyl Phthalate (DOP), which has a large number of 0.3  $\mu\text{m}$  droplets, into the upstream side of the filter during operation. Readings are taken on the opposite side of the filter to quantify the number of droplets that penetrate. Thus, if a filter allows one or less droplet to penetrate the filter with an initial concentration of 10,000, the filter is rated at 99.99% efficiency.

As most aerosol droplets are larger than 0.3  $\mu\text{m}$ , the collection efficiency of HEPA filters for these droplets is actually higher than its rating. Variations in filter efficiency, for example from 99.95% to 99.99%, are usually due to manufacturing techniques.

The life of HEPA filters varies greatly with the hours of operation, the cleanliness of the laboratory, and the nature of the work being done. With typical usage, however, HEPA filters commonly last from three to five years before needing replacement.

## Class I Cabinet

The Class I cabinet is defined as a ventilated cabinet for personnel and environmental protection, with unrecirculated airflow away from the operator. Class I cabinets have a similar airflow pattern to a fume hood, except that the Class I cabinet has a HEPA filter at the exhaust outlet, and it may or may not be connected to an exhaust duct system (Figure 5). The Class I cabinet operates with an inflow or face velocity of 75 to 100 feet per minute (fpm).

Class I cabinets are suitable for work with agents that require **Biosafety Level 1, 2 or 3 containment**.

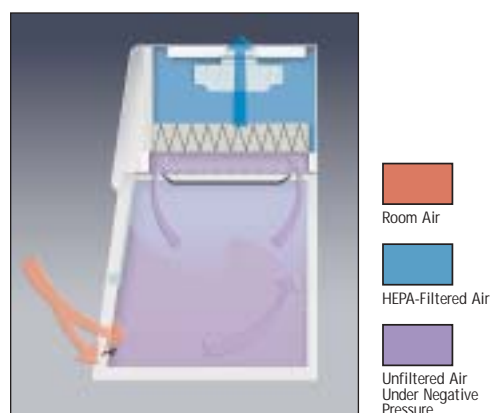


Figure 5. The Class I Cabinet

The major disadvantage of the Class I Cabinet is that it offers no product protection from contamination and therefore is very limited in its application.

## Class III Cabinets

The Class III cabinet is defined as a totally enclosed, ventilated cabinet of leak-tight construction. Operations in the cabinet are conducted through attached rubber gloves. The cabinet is maintained under negative air pressure of at least 0.5 inch (12.7 mm) water gauge (w.g.). Supply air is drawn into the cabinet through HEPA filters. The exhaust air is treated by double HEPA filtration, or by HEPA filtration and incineration (Figure 6). The cabinet also has a transfer chamber capable of sterilizing work materials before exiting the glove box containment system. Class III cabinets are suitable for work with agents that require **Biosafety Level 1, 2, 3 or 4 containment**.

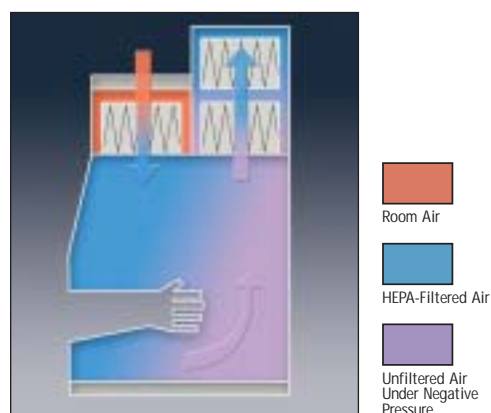


Figure 6. The Class III Cabinet

## Class II Cabinets

The Class II cabinet is defined as a ventilated cabinet for personnel, product and environmental protection, having an open front with inward airflow for personnel protection, downward HEPA-filtered laminar airflow for product protection, and HEPA-filtered exhaust air for environmental protection. Class II cabinets are suitable for use with agents that require Biosafety Level 1, 2 or 3 containment. The cabinets are differentiated into various types based on their construction, airflow velocities and patterns, and by their exhaust system (Table 3).

### Types of Class II Cabinets

The primary design differences between Type A and Type B Class II cabinets are: Type A1 cabinets may have contaminated plenums under positive pressure that are exposed to the room; Type A2 and B cabinets must surround all contaminated positive pressure plenums with negative pressure ducts. The minimum

Type	A1		A2		B1	B2
Minimum average inflow velocity (fpm)	75		100		100	100
Percentage air recirculated (approximate)	70		70		30-50	0
Exhausts to	Room	Outside	Room	Outside	Outside	Outside
Exhaust duct connection	None	Canopy	None	Canopy	Hard Duct	Hard Duct
Requires dedicated exhaust connection	No	No	No	No	Yes	Yes
Requires face velocity alarm	No	No	No	No	Yes	Yes
Suitable for work with odorous materials	No	Yes	No	Yes	Yes	Yes
Suitable for work with toxic chemicals and radionuclides	No	No	No	Minute Quantities	Minute Quantities	Minute Quantities
Capital cost	Low	Moderate	Low	Moderate	High	High
Installation cost	Low	Moderate	Low	Moderate	High	High
Energy loss	Low	Moderate	Low	Moderate	High	High

Table 3. Class II Cabinet Characteristics

average face velocity for Type A1 is 75 fpm; Type A2 and B is 100 fpm. Type A cabinets intermix and recirculate some (approximately 70%) of their air after HEPA filtration; Type B cabinets directly exhaust (30-50% for Type B1) or all (Type B2) of their air out of the laboratory after HEPA filtration. Type A cabinets are exhausted into the lab or outside via a canopy connection; Type B must have a dedicated, sealed exhaust system with remote blower and appropriate alarm system.

### Type A1 (formerly Type A)

Type A1 cabinets (1) maintain minimum average inflow velocity of 75 fpm through the work area access opening; (2) have HEPA-filtered downflow air that is a portion of the mixed downflow and inflow air from a common plenum (i.e. plenum from which a portion of the air is exhausted from the cabinet and the remainder supplied to the work area); (3) may exhaust HEPA-filtered air back into the laboratory or to the environment through an exhaust canopy; and

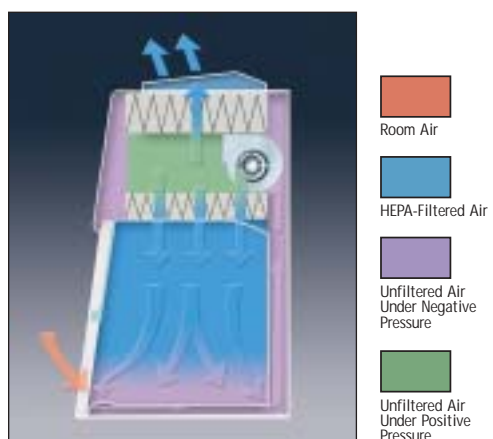


Figure 7. The Class II, Type A1 Cabinet

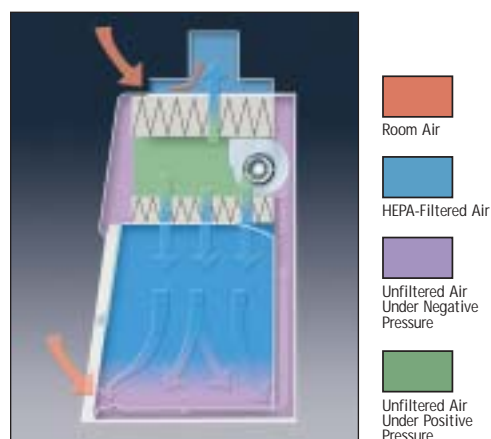


Figure 8. The Class II, Type A1 Cabinet with Canopy-Type Connection



(4) may have positive pressure contaminated ducts and plenums that are not surrounded by negative pressure plenums. Type A1 cabinets are suitable for work with biological agents in the absence of volatile toxic chemicals and volatile radionuclides.

The Class II, Type A1 cabinet may exhaust HEPA-filtered air back into the laboratory (Figure 7). A Type A1 cabinet may also be exhausted to the outside via a canopy-type connection to remove non-toxic odors (Figure 8). In this installation, the cabinet exhausts its HEPA-filtered air and a portion of the laboratory's air to the outside.

### Type A2 Cabinet (formerly Type A/B3)

Type A2 cabinets (1) maintain a minimum average inflow velocity of 100 fpm through the work access opening; (2) have HEPA-filtered downflow air that is a portion of the mixed downflow and inflow air from a common exhaust plenum; (3) may exhaust HEPA-filtered air back into the laboratory or to the environment through an exhaust canopy; and (4) have all biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums. Type A2 cabinets with a canopy are suitable for work with biological agents treated with minute quantities of toxic chemicals and tracer quantities of radionuclides that will not interfere with the work if recirculated in the downflow air.

### Type B1 Cabinet

Type B1 cabinets (1) maintain a minimum average inflow velocity of 100 fpm through the work access opening; (2) have HEPA-filtered downflow air composed largely of uncontaminated recirculated inflow air; (3) exhaust most of the contaminated downflow air through a dedicated duct exhausted to the atmosphere after passing through a HEPA filter; and

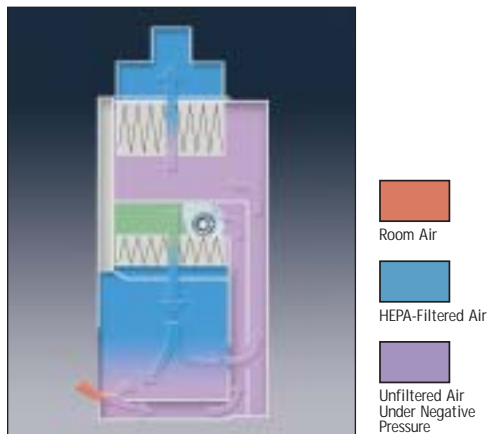


Figure 9. The Class II, Type B1 Cabinet

(4) have all biologically-contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums (Figure 8).

Type B1 cabinets are suitable for work with agents treated with minute quantities of toxic chemicals and tracer amounts of radionuclides required as an adjunct to microbiological studies if work is done in the directly exhausted portion of the cabinet, or if the chemicals or radionuclides will not interfere with the work when recirculated in the downflow air.

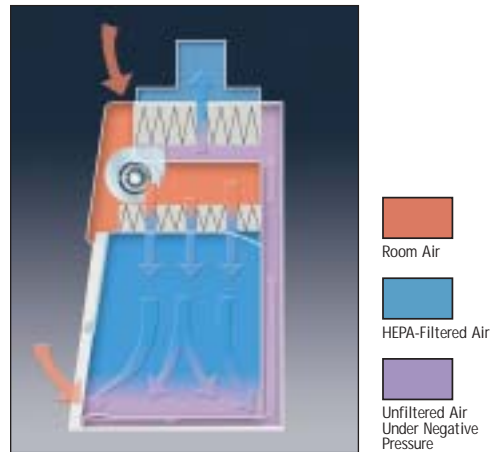


Figure 10. The Class II, Type B2 Cabinet

### Type B2 Cabinet

Type B2 cabinets, which are sometimes referred to as "total exhaust," (1) maintain minimum average inflow velocity of 100 fpm through the work area access opening; (2) have HEPA-filtered downflow air drawn from the laboratory or the outside air (i.e. downflow air is not recirculated from the cabinet exhaust air); (3) exhaust all inflow and downflow air to the atmosphere after filtration through a HEPA filter without recirculation in the cabinet or return to the laboratory; and (4) have all contaminated ducts and plenums under negative pressure or surrounded by directly exhausted (nonrecirculated through the work area) negative pressure ducts and plenums (Figure 9). Type B2 cabinets may be used with biological agents treated with toxic chemicals and radionuclides required as an adjunct to microbiological studies.

## NSF Standard Number 49

In the late 1960's to mid 1970's, researchers often submitted their own Class II cabinet specifications and options to manufacturers. In order to standardize cabinet design and performance used by governmental researchers, the National Institutes of Health



*The NSF International mark appears on Class II Cabinets that are listed by NSF International*

developed a specification for Type A cabinets in 1973. The National Cancer Institute also developed its own specifications for a Type B1 cabinet the same year. The government specifications were demanding ones, and often an impediment to development by the manufacturers. In an attempt to develop impartial specifications, NIH contacted the National Sanitation Foundation (NSF International) in the early 1970's. NSF International is an independent organization that acts as a neutral agency serving the consumer, government and industry in developing solutions for problems pertaining to public health and the environment. After numerous meetings with government officials, scientists, and manufacturers, NSF International published NSF Standard 49 in 1976.

The NSF/ANSI Standard 49, entitled Class II (Laminar Flow) Biosafety Cabinetry, establishes minimum materials, design, construction and performance requirements for Class II biosafety cabinets, the quality control tests that the manufacturer must perform on every unit, and the tests that should be performed in the field. The Standard also provides the Type designations, which were described above, that further delineate the various cabinets based on airflow characteristics. NSF International periodically reviews and changes the Standard as needed and, in 2002, updated its Standard with new Type designations.

Its policy establishes requirements for testing and periodic retesting of cabinets by the NSF International Laboratory in Ann Arbor, Michigan, and annual unannounced audits of the manufacturer's facility. The Standard requires three separate biological challenge tests to be passed in order for the cabinet to be listed. The Personnel Protection Test measures the number of bacterial spores escaping from the cabinet's work area into the environment. The Product Protection Test establishes the number of bacterial spores entering the work area from the outside environment. Finally, the Cross Contamination Protection Test measures how far bacterial spores can drift across the cabinet work area.

Performance Zone Tests are additional biological challenges performed at various inflow and downflow velocity settings. These tests ensure that the cabinet still functions properly, even when it is not operating at the manufacturer's recommended downflow and inflow velocity settings.

These tests are performed by NSF International before a cabinet is listed, and once every five years thereafter. Typically, most manufacturers perform biological challenge tests while their cabinets are in the prototype stage of development, and periodically during production, to ensure that the cabinets protect personnel, environment and product.

## Selecting the proper Biosafety Cabinet

### Primary Consideration—Safety

Selecting the proper type of biosafety cabinet depends on the following: (1) the type of protection required—a) product protection only b) personnel and environmental protection only c) product, personnel, and environmental protection, (2) the different types of work that will be done in the cabinet, (3) the types and quantities of toxic materials that will be used in the course of the work, (4) the type of exhaust system that will be needed. Items 1, 2 and 3 are all determined by the user of the cabinet, based on his/her individual needs. Item 4 will be determined by the first three. Biological containment is not an issue - ALL Class II cabinets are designed for Biosafety Levels 1, 2 and 3 containment. No one type offers superior aerosol containment over the others.

### Secondary Consideration—Cost

Understanding the costs involved in owning and operating a biosafety cabinet is important. Capital expenditures include the cabinet, the remote blower, the ductwork and other hardware such as alarms and dampers. Installation outlays involve installation of the cabinet, the exhaust systems and its blower, and initial certification. Finally, maintenance costs including recertification and HEPA filter replacement are considerations that should not be overlooked.

### Tertiary Considerations—Ergonomics, ADA-Compliance and Options

Ergonomic features of a biological safety cabinet are important considerations because they can affect the safety and comfort of the user. When monitors and controls are in close proximity to the user's field of vision or reach, the user experiences less fatigue and reduced risk of personal injury. Low-mounted features comply with the Americans with Disabilities Act, essential for wheelchair-bound users. An angled front sash allows closer viewing without glare. A contoured

air foil permits the user's forearms to rest comfortably. Accessories that contribute to the user's comfort such as an adjustable, ergonomic chair and footrest should be considered. Supporting base stands that may be adjusted with a hydraulic lift or hand crank may be beneficial if several users of differing body types will be using the cabinet. Ensure that adjustable height base stands meet NSF standards for tip resistance and construction stability.

Many options and accessories are available that customize a biosafety cabinet to the user's needs. Cabinets come in various widths. The most common outside widths of biosafety cabinets are 3-, 4- and 6-feet. The three foot wide cabinet offers the greatest savings of money and bench space, however, many researchers find the limited work space too confining. The four foot wide cabinet is by far the most popular, offering a larger work area, at a slightly higher cost. The six foot wide cabinet offers room for two researchers to work side-by-side, however, its size and weight may make some installations difficult.

If the user is confused about which size is needed, it is useful to mark a section of laboratory benchtop space equal to the dimensions of the cabinet's work area. The researcher should then perform several "dry runs" of his/her work within the marked area, to see if it is large enough.

Service valves are accessories that many researchers prefer to have on their cabinets. The valves, which should be easily accessible to the operator, may provide water, vacuum, air or a variety of other gases. The use of flammable gases or solvents in biosafety cabinets should be avoided. Open flames and gases under high pressure should not be used because they disrupt the airflow patterns in the cabinet. Open flames may also burn the HEPA filter and/or damage the filter's adhesive.

As with service valves, many researchers prefer the convenience of electrical outlets on the biosafety cabinet. The outlets should be factory installed with a dedicated circuit breaker, so that a short circuit would not prevent the cabinet from operating. The outlets and their breakers should be rated for the requirements of the user, and as with the valves, they should be easily accessible. Ground fault interrupter circuits (GFI) may be considered.

Ultraviolet (UV) lamps are often installed in a biosafety cabinet as an aid in decontamination of the work area. The lamps are similar in construction to fluorescent lights, except they emit ultraviolet light with a wavelength of 254 nanometers (nm). This wavelength of light is disruptive to DNA molecules,

resulting in a broad spectrum disinfection. While UV light is effective when it strikes a microbial cell directly, it is ineffective when the cell is protected by dust, dirt, or organic matter. UV irradiation of the work area should only be used as a supplemental method of maintaining the disinfected status of the cabinet; it should never be relied on alone to disinfect a contaminated work area. Ultraviolet irradiation is irritating to the eyes, and the UV lamp should never be on when using the cabinet.

An intravenous (IV) bar is simply a rod suspended across the top of the work area, from which the technician can hang IV bags or bottles during their preparation. Placing an IV bar in the work area disrupts the airflow patterns in the cabinet, so the user must be sure that the cabinet has passed all NSF/ANSI Standard 49 tests with the bar in place.

For benchtop-style biosafety cabinets, a base stand is a necessary accessory. The base stand must pass NSF/ANSI Standard 49 stability tests with the cabinet attached in order to be used with the cabinet.

## Installation

The cabinet should be located away from fans, heating and air conditioning registers, laboratory hoods, high traffic areas and doors that could interfere with its airflow patterns (Figure 12). All windows in the room should remain closed. The floor should be level, and of solid construction. A dedicated electrical outlet should also be available.

There should be a minimum clearance of 6" between the exhaust outlet on the top of the cabinet and any overhead obstructions. Whenever possible, a minimum 12" clearance should be provided at the back and both sides of the cabinet.

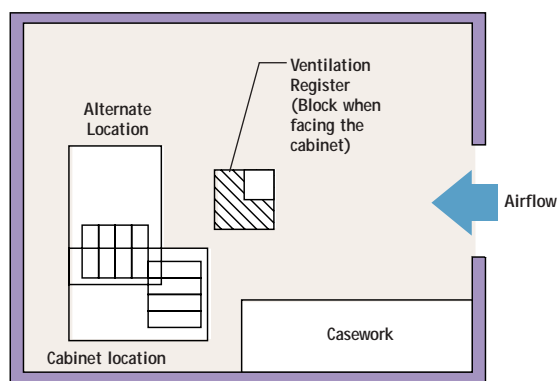


Figure 12. A Typical Class II Cabinet Installation

Certification

Certification of biosafety cabinets plays a critical role in their performance. Certification should be performed at least annually and whenever the cabinet is moved, serviced or when the HEPA filters are replaced. The recommended tests in Table 4 and their procedures are outlined in NSF/ANSI Standard 49.

Selecting the right certifier is critical to ensure that the biosafety cabinet performs as it was designed. The efforts of the manufacturer's research and development, and NSF's testing and validation can easily be nullified by an improper or inaccurate certification.

NSF International and other agencies offer accreditation programs for biosafety cabinet certifiers. Upon successful completion of written and practical examinations by NSF, the certifier is accredited to certify biosafety cabinets using test methods described in NSF/ANSI Standard 49.

Downflow Velocity Profile	Establishes the average downflow velocity of the cabinet.
Inflow Velocity	Establishes the average inflow velocity of the cabinet.
Airflow Smoke Pattern Test	Establishes the airflow patterns around the work access opening and within the work area.
HEPA Filter Leak Test	Tests the HEPA filters for leaks.
Vibration Test	Measures the vibration of the cabinet during operation.
Noise Level Test	Measures the sound pressure of the cabinet during operation.
Lighting Intensity Test	Establishes the light intensity in the work area.

Table 4. Recommended Certification Tests

If the certifier is not accredited by NSF, then the following should be considered: The user should ask the certifier about his experience certifying biosafety cabinets in general, and the user's model specifically. The user should ask the certifier about his attendance at technical courses or the manufacturer's training sessions. The certifier should describe all the tests he will perform and his procedures. Any deviations from NSF/ANSI Standard 49 should be explained and understood before the certification begins. The certifier's test equipment should be annually calibrated to a National Institute of Standards & Testing (NIST) traceable standard.

Special Applications for Biosafety Cabinets

Handling Suspicious Mail

Bioterrorism involving mail potentially contaminated with biohazards such as anthrax and smallpox has created demand for enclosures where suspicious mail can be safely sorted and opened. The Centers for Disease Control and Prevention (CDC) recommends handling anthrax and smallpox using Biosafety Level 2 practices, containment equipment and facilities. The Biosafety Level 2 recommendation is required for work on a laboratory scale, which would be similar to the small amounts of infected agents found in dangerous mail. Class I enclosures are Biosafety Level 2 equipment that provide the necessary personnel protection without providing unneeded product protection. Class I enclosures, like any laboratory device, have the potential for misuse by persons unfamiliar with biological safety cabinets or common laboratory practices such as aseptic technique. Training of personnel is essential and the risk of misuse must be weighed against the potential for exposure to biohazards. Consult your safety officer for recommendations based on your specific application.

Handling cytotoxic or hazardous drugs

Many drugs currently in use may be oncogenic, mutagenic, or exert any number of toxic side effects on health care professionals exposed to them. Aerosols or chemical dusts are often generated during routine handling and preparation of these hazardous drugs. The American Society of Health-System Pharmacists (ASHP) recommends, as a minimum measure, that work involving hazardous drugs be performed in a Class II, Type A1 or A2 Cabinet (Figure 7). The selection of a hard-ducted Type B2 cabinet is preferable. The cabinet should be operated 24 hours a day, seven days a week. Proper manipulative technique must be taught to all workers. The cabinet should be surface decontaminated at least weekly, or whenever there is a spill. The cabinet should be certified as per NSF/ANSI Standard 49 every six months. For current ASHP guidelines, visit [www.ashp.org/bestpractices](http://www.ashp.org/bestpractices) and click on ASHP Technical Assistance Bulletins.

Working with clinical material

The Centers for Disease Control and the National Institutes of Health have recommended Universal Blood and Body Fluid Precautions or Universal Precautions. Under these precautions, blood and certain body fluids of all patients are considered potentially infectious for Human Immunodeficiency Virus (HIV), hepatitis B virus (HBV), and other bloodborne pathogens.

Recommended biosafety levels, facility requirements, and the classification of many agents are included in the CDC-NIH publication *Biosafety in Microbiology and Biomedical Laboratories*, U.S. Department of Health and Human Services, HHS publication (CDC) 99-8395, 4th edition, April 1999. The publication is available for downloading from CDC's website at [www.cdc.gov](http://www.cdc.gov). CDC/NIH recommended precautions for laboratory work with HIV follow: (Table 5).

Activities Involving:	Practices & Procedures	Facility
clinical specimens/body fluids	Biosafety Level 2	Biosafety Level 2
human/animal tissues infected with HIV, growing HIV at research lab scale, growing HIV-producing cell lines, working with concentrated HIV preparation, droplet/aerosol production	Biosafety Level 3	Biosafety Level 2
HIV at industrial-scale levels, large volume, or high concentration production and manipulation	Biosafety Level 3	Biosafety Level 3

Table 5. CDC/NIH Recommended Precautions for Laboratory Work with HIV

Use of the Cabinet

Planning

Thoroughly understand procedures and equipment required before beginning work.

Arrange for minimal disruptions, such as room traffic or entry into the room, while the cabinet is in use.

Start-Up

Turn off UV light if in use. Ensure that the sash is set in the correct operating position.

Turn on fluorescent light and cabinet blower.

Check the return air grilles for obstructions, and note the pressure gauge reading.

Allow the cabinet to operate unobstructed for fifteen minutes.

Wash hands and arms thoroughly with germicidal soap.

Wear a long sleeved lab coat with knit cuffs and over-the-cuff rubber gloves. Use protective eyewear. Wear a protective mask if appropriate.

Wipe-Down

Wipe down the interior surfaces of the cabinet with 70% ethanol, or a suitable disinfectant, and allow to dry.

Loading Materials and Equipment

Only load the materials required for the procedure. Do not overload the cabinet.

Do not obstruct the front, side, or rear return air grilles. Large objects should not be placed close together.

After loading the cabinet, wait two to three minutes to purge airborne contaminants from the work area.

Work Techniques

Keep all materials at least four inches inside the sash, and perform all contaminated operations as far to the rear of the work area as possible.

Segregate all clean and contaminated materials in the work area.

Arrange materials to minimize the movement of contaminated materials into clean areas.

Keep all discarded contaminated material to the rear of the cabinet.

Avoid moving materials or excessive motion of the operator's hands and arms through the front access opening during use.

Avoid the use of an open flame.

Use proper aseptic technique.

Avoid using techniques or procedures that disrupt the airflow patterns of the cabinet.

If there is a spill or splatter during use, all objects in the cabinet should be surface decontaminated before removal. Thoroughly disinfect the working area of the cabinet WHILE IT IS STILL IN OPERATION. This will prevent the release of contaminants from the cabinet.

Final Purging

Upon completion of work, the cabinet should be allowed to operate for two to three minutes undisturbed, to purge airborne contaminants from the work area.

Unloading Materials and Equipment

Objects in contact with contaminated material should be surface decontaminated before removal from the cabinet.

All open trays or containers should be covered before being removed from the cabinet.

Wipe-Down

Wipe down the interior surfaces of the cabinet with 70% ethanol, or a suitable disinfectant, and allow to dry.

Periodically remove the work surface and wipe down the area beneath it.

Dispose of rubber gloves appropriately, and have the lab coat laundered properly.



## Shutdown (Optional)

Turn off the fluorescent light and cabinet blower, if desired, and turn on the UV light if appropriate.

## Routine Maintenance Schedule

Under normal operation, biosafety cabinets require little routine maintenance. The following schedule is recommended:

### Weekly

Using 70% ethanol, or a suitable disinfectant, surface disinfect the inside of the cabinet and the work surface.

Using an appropriate glass cleaner, clean the sash and the surface of the UV lamp, if so equipped.

Operate the cabinet blower, noting the pressure gauge reading in the operational log.

### Monthly (or more often as required)

Using a damp cloth, clean the exterior surfaces of the cabinet, particularly the front and the top of the cabinet, to remove any accumulated dust.

Disinfect and remove the work surface. Surface disinfect the lower plenum with a solution of 70% ethanol, or a suitable disinfectant.

Check all service valves, if so equipped, for proper operation.

### Annually

Have the cabinet recertified by a qualified certification technician.

Replace the UV lamp, if so equipped.

### Biannually

Replace the fluorescent lamp.

## Glossary

**absolute filter:** Obsolete term for HEPA filter.

**aerosol:** A colloid of liquid or solid particles suspended in a gas, usually air.

**agent:** Any biological, chemical or physical power, principle or substance capable of acting upon a subject organism, usually to its detriment.

**air barrier ("air curtain"):** The unidirectional movement of air past and parallel to the plane of an opening and at a velocity greater than that on either side, thereby creating an impedance to transverse movement of airborne particulates through the opening.

**air stream:** A current of air; airflow.

**antiseptic:** A compound that prevents the multiplication of microorganisms. Bacteriostatic in action, not bactericidal. Its use applies to tissues rather than inanimate surfaces.

**aseptic technique:** The performance of a procedure or operation in a manner that prevents the introduction of microorganisms, which are capable of causing infection or contamination.

**assessment of risk:** The process of defining biological hazard associated with a microbial or antigenic entity.

**biohazard:** A contraction of the words biological and hazard; infectious agent(s) presenting a real or potential risk to the well-being of man, other animals, or plants, either directly through infection or indirectly through disruption of the environment.

**biosafety cabinet:** See biological safety cabinet.

**biological challenge:** A series of tests performed to assure that aerosols are contained within the cabinet, that outside contaminants do not enter the cabinet, and contaminants in the cabinet remain localized. Suspensions of *Bacillus subtilis subsp. niger* spores are used as an indicator in the tests.

**biological safety cabinet:** Cabinet intended to protect the user and environment from the hazards of handling infectious material and other biohazardous material. Some types may also protect the materials being handled in them from contamination.

**Brownian motion:** A random movement of microscopic particles suspended in liquids or gases resulting from impact of the molecules of the suspending agent on the particles.

**canopy connection:** A biosafety cabinet exhaust system with a physical gap or space between the cabinet's exhaust and the exhaust system intake. During operation, the exhaust system draws all of the cabinet's exhaust air through the duct, plus a small volume of room air through the gap.

**certification:** When pertaining to safety cabinets, measurement and/or correction of a safety cabinet's air velocities, balance and filtration system integrity by a qualified technician.

**chemical carcinogen:** Those chemicals designated as posing a potential occupational carcinogenic risk to workers by OSHA or the Department of Health and Human Services Committee for Coordinating Toxicology and Related Programs.

**clean room:** A dust-free facility.

**collection efficiency:** Usually expressed as the percentage of material collected compared with the total amount present, it may be calculated on a particle number basis or a total weight basis.

**contamination:** Any foreign substance that makes an unwanted incursion. In the present context, usually viable airborne particulates.

**decontamination:** The destruction or removal of living organisms (this does not imply either total destruction or total removal), or the removal or neutralization of toxic agents or chemical carcinogens; to make an object safe for unprotected individuals.

**di- (2 ethyl hexyl) phthalate:** See DOP.

**di-sec-octyl phthalate:** See DOP.

**diffuser:** A device, often a screen, used to distribute airflow evenly.

**diffusion:** A phenomenon of HEPA filtration by which Brownian motion causes particles to diffuse across air stream lines impacting them on a filter fiber.

**dioctyl phthalate:** See DOP.

**disinfectant:** A chemical agent that kills or inactivates vegetative bacteria, fungi, and viruses, but not necessarily spores. This term applies to inanimate surfaces as opposed to tissues.

**DOP:** Dioctylphthalate, an oil that can be aerosolized to an extremely uniform size; i.e. 0.3  $\mu\text{m}$  for a major portion of any sample; the aerosol is used to challenge HEPA filters.



**hot DOP:** Produced by controlled vaporization and condensation of liquid DOP to give a cloud of monodisperse droplets with diameters of approximately 0.3  $\mu\text{m}$ .

**cold DOP:** Produced by compressed air atomization of room temperature liquid DOP, aerosol size 0.3 to 3.0  $\mu\text{m}$  with a mean diameter of 0.7  $\mu\text{m}$ .

**downstream:** In the direction of the flow.

**droplet:** An airborne particle consisting primarily of liquid. While some settle out quickly, many dry to become droplet nuclei and can add significant numbers of microorganisms to the air.

**exhaust:** The withdrawing and expelling of air from the cabinet by means of a blower or fan; that portion of the cabinet air that is discharged after filtration, either to the room or into a ventilation system.

**filter:** A device used for removal of particulates, including microorganisms, from air or other gases. (Also see HEPA filter.)

**filter efficiency:** The efficiency of various filters can be established on the basis of entrapped particles, i.e., collection efficiency; or on the basis of particles passed through the filter, i.e., penetration efficiency.

**germicidal:** Able to destroy bacteria, fungi, viruses and other similar organisms.

**hard-ducting:** Permanently installed airtight ductwork not intended to be disassembled for normal cabinet servicing or testing.

**HEPA filter:** High-efficiency particulate air filter. A disposable extended-pleated dry-type filter with (1) a rigid casing enclosing the full depth of the pleats; (2) a minimum particle removal efficiency of 99.9% for thermally generated monodisperse DOP smoke particles with a diameter of 0.3  $\mu\text{m}$ ; and (3) a maximum pressure drop of 1 inch water gauge when clean and operated at its rated airflow capacity.

**high efficiency particulate air filter:** See HEPA filter.

**horizontal laminar flow bench:** A ventilated cubicle with solid sides having a table-height work surface and unidirectional, minimum turbulence air entering from a vertically mounted high efficiency filter at one side and leaving the cubicle at the opposite (open) side.

**inches of water gauge (in w.g.):** A unit of pressure equal to the weight of a column of liquid water one inch high at 20°C (1 in. w.g. = 0.036 psi).

**infectious agent:** As used in this text, agents capable of producing a disease or abnormal response in man, laboratory animals, or a tissue culture system.

**inflow velocity:** Air velocity at the cabinet's work opening; velocity of the air entering the cabinet at the work opening.

**laminar airflow:** Airflow in which the entire body of air within a designated space moves with uniform velocity along parallel flow lines.

**monodisperse aerosol:** An aerosol containing particles of nearly the same size.

**negative pressure:** Pressure in a space that causes an inflow of air.

**partial containment enclosure:** An enclosure that is constructed so that contamination between its interior and the surroundings is minimized by the controlled movement of air. Class I and Class II safety cabinets are examples.

**plenum:** An enclosure for flowing gases in which the static pressure at all points is relatively uniform.

**positive pressure:** Pressure in a space that causes an outflow of air.

**protection:** In Class II cabinets, any aerosol generated is kept away from the technician doing the work.

**environmental protection:** Any aerosol generated within the cabinet is removed from the air or deactivated (such as by incineration) before the air from the cabinet is discharged either inside or outside the facility.

**personnel protection:** Any aerosol generated within the cabinet is kept away from the technician doing the work.

**product protection:** The air at the work surface of the cabinet has been filtered so that it is free of airborne particles and organisms that could contaminate the work.

**static pressure:** The pressure of a fluid exerted in all directions equal and opposite to the pressure tending to compress the fluid. In ventilation applications, static pressure is usually the difference between the absolute pressure in an exhaust system and atmospheric pressure.

**sterile:** The absence of all life on or in an object. This is an absolute term; there can be no such description as nearly sterile, partially sterile, etc.

**sterilize:** Any process, physical or chemical, which results in the absence of all life in an object, applied especially to microorganisms, including bacteria, fungi, and their spores and the inactivation of viruses.

**supply air:** Air entering the cabinet through the work opening to make up for the volume of air exhausted.

**thimble connection:** See canopy connection.

**ultraviolet (UV) light:** Radiation in the electromagnetic spectrum having wavelengths from approximately 200 to 390 nanometers.

**velocity:** The time rate of linear motion in a given direction.

**capture and/or containment velocity:** The velocity necessary to capture or contain a generated contaminant, in a cabinet this usually ranges from 50 to 200 fpm.

**viable:** Literally, capable of life. Generally refers to the ability of microbial cells to grow and multiply as evidenced by formation of colonies on an agar culture medium; or, as with viruses, to divert the host cell's metabolism to replication of the parasite.

**virus:** A parasitic microorganism, smaller than a bacterium. Viruses have no independent metabolic activity, and may replicate only within a cell of a living plant or animal host.

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