Primary Containment for Biohazards:

Selection, Installation and Use of Biological Safety Cabinets

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SECTION I

Introduction

This text presents information on the design, selection, function and use of biological safety cabinets (BSCs), which are the primary means of containment developed for working safely with infectious microorganisms. Brief descriptions of the facility and engineering concepts for the conduct of microbiological research are also provided. BSCs are only one part of an overall biosafety program which requires consistent use of good microbiological practices. Detailed descriptions of acceptable work practices, procedures, and facilities, described as biosafety levels 1 through 4, are presented in the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*[6].

BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of biological safety cabinets, designated as Class I, II and III have been developed to meet varying research and clinical needs.

High efficiency particulate air (HEPA) filters or ultra-low penetration air (ULPA) filters are used in the exhaust and/or supply systems of biological safety cabinets These filters and their use in BSCs are briefly described in Section II. Section III presents a general description of the special features integrated into biological safety cabinets to provide varying degrees of personnel, product and environmental protection.

Laboratory hazards and risk assessment are discussed in Section IV. Section V presents the laboratorian with work practices, procedures and practical tips to maximize the protection afforded by the most commonly used BSCs. Facility and engineering requirements needed for the operation of each type of BSC are presented in Section VI. Finally, Section VII reviews some of the requirements for routine annual certification of cabinet operation and integrity.

These sections are not meant to be definitive or all-encompassing. Rather, an overview is provided to clarify the expectations, functions and performance of these critical primary barriers. This document has been written for the laboratorian, engineer, manager, or procurement officer who desires a better understanding of each type of cabinet and the rationale for selecting the appropriate BSC to meet specific operational needs.

SECTION II

The High Efficiency Particulate Air (HEPA) Filter and the Development of Biological Containment Devices

From the earliest laboratory-acquired typhoid infections to the hazards posed by today's antibiotic-resistant bacteria and rapidly-mutating viruses, threats to worker safety have stimulated the development and refinement of cabinets in which infectious microorganisms could be safely handled. Work with cell cultures, the need to maintain sterile cell lines, and the need to minimize cross-contamination to maintain product integrity were also addressed in the design of cabinets.

The use of proper microbiological procedures, aseptic techniques, and equipment (as described in *BMBL*)[6] cannot be overemphasized in providing primary personnel and environmental protection. For example, high-speed blenders designed to reduce aerosol generation, needle-locking syringes, microburners, and safety centrifuge cups or sealed rotors are among the engineering devices that protect the laboratorian. However, the most essential piece of containment equipment is the biological safety cabinet in which manipulations of microorganisms are performed.

Background

Early prototype clean air cubicles were designed to protect the materials being manipulated from contamination (e.g., from the room or from the worker), rather than to protect the worker from the risk of manipulating the materials. Filtered air was blown across the work surface directly at the worker. Therefore, these cubicles could not be used for handling infectious agents, because the worker would be in a contaminated air stream.

To protect the worker during manipulations of infectious agents, a small workstation was needed that could be installed in existing laboratories with a minimum of modification to the room. The earliest designs for primary containment devices were essentially non-ventilated "boxes" built of wood and later of stainless steel, within which simple operations such as weighing materials could be accomplished.[16]

Early versions of ventilated cabinets did not have adequate and controlled directional air movement, and were characterized by mass air flow with widely varying air volumes across openings. The feature of mass air flow into the cabinet was added to draw "contaminated" air away from the laboratorian. This was the forerunner to the Class I BSC. However, since it was unfiltered, the room air drawn into to the cabinet contained environmental microorganisms and other undesirable particulate matter.

HEPA Filters

Control of airborne particulate materials became possible with the development of filters which would efficiently remove microscopic contaminants from the air. The high efficiency particulate air (HEPA) filter was developed to create dust-free work environments (e.g., "clean rooms" and "clean benches") in the 1940's.[16]

HEPA filters are generally rated as being effective at removing 0.3μm-sized particles with an efficiency of at least 99.97%; they are even more effective at removing both smaller and larger particles.[16,24] A detailed explanation of HEPA filter efficiency and the mechanics of particle collection have been well documented[9,18] and only a brief description is included here.

The medium of a typical HEPA filter is a single sheet of borosilicate fibers which has been treated with a wet-strength water-repellant binder. The filter medium is pleated to increase the overall surface area inside the filter frame, and the pleats are often divided by corrugated aluminum separators (Figure 1). These prevent the pleats from collapsing in the air stream and provide a path for air flow. The filter is glued into a wood, metal, or plastic frame. Careless handling of the filter (e.g., improper storage or dropping) can damage the medium at the glue joint and cause tears or shifting of the filter which result in leaks in the medium. This is the primary reason why filter integrity must be certified after a BSC is initially installed and after it has been relocated (see Section VII).

Various types of containment devices incorporate the use of HEPA filters in the exhaust or supply air system to trap airborne particulate material. Depending on the configuration of these filters and the direction of airflow, varying degrees of personnel, environmental and product protection can be achieved.[26] Section V describes good practices and procedures to be followed in order to address these safety concerns.

SECTION III

Biological Safety Cabinets

The similarities and differences in protection offered by the various classes of biosafety cabinets are reflected in Table 1. Please also refer to Table 2 and Section IV for further considerations pertinent to BSC selection and risk assessment.

The Class I BSC

The Class I BSC provides personnel and environmental protection, but no product protection. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment (Figure 2). 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[5] through the front opening. Because of the product protection provided by the Class II BSCs, general usage of the Class I BSC has declined. However, in many cases Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures (e.g. cage dumping, aerating cultures or homogenizing tissues) with a potential to generate aerosols that may flow back into the room.

The Class I BSC is hard-ducted to the building exhaust system, thimble-connected, or recirculated back into the room depending on use. If it is hard-ducted, the building exhaust fan provides the static pressure necessary to draw room air into the cabinet. Cabinet air is drawn through a HEPA filter as it enters the exhaust plenum. Sometimes a second HEPA filter is installed in the building exhaust system.

A steel panel with 8" arm holes to allow access to the work surface can be added to the Class I cabinet. The restricted opening results in increased inward air velocity, thereby increasing worker protection. For added safety, arm-length gloves can be attached to the panel. Makeup air is then drawn through an auxiliary air supply opening (which may contain a filter) and/or around a loose-fitting front panel. To permit access to the cabinet interior with the panel installed, a double-door air lock is attached on either side of the cabinet. Consideration must be given to the chemicals used in a BSC with HEPA filters as some chemicals can destroy the filter medium, housings and/or gaskets causing the loss of containment.

The Class II BSC

As biomedical researchers began to use sterile animal tissue and cell culture systems, particularly for the propagation of viruses, cabinets were needed that also provided product protection. In the early 1960's, a principle evolved stating that unidirectional air moving at a steady velocity along parallel lines (i.e., "laminar flow") would aid in the capture and removal of airborne contaminants.[31] Biocontainment technology also incorporated this laminar flow principle with the use of the HEPA filter to provide a particulate-free work environment. This combination serves to protect the laboratorian from the potentially infectious microorganisms being manipulated[18] and provid necessary product protection.

The Class II (Types A, B1, B2, and B3)[24] biological safety cabinets provide personnel, environmental and product protection. Air flow is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air exhaust is passed through a certified exhaust HEPA filter, it is contaminant-free (environmental protection), and may be recirculated back into the laboratory (Type A BSC) or exhausted out of the building (Type B BSC).

HEPA filters are effective at trapping particulates and infectious agents, but not at capturing volatile chemicals or gases. Only BSCs that are exhausted to the outside should be used when working with volatile toxic chemicals (see Table 2). In certain cases a charcoal filter may be added to prevent release of toxic chemicals into the atmosphere.

Biological Safety Cabinets

All Class II cabinets are designed for work involving microorganisms assigned to biosafety levels 1, 2 and 3.[6] Class II cabinets provide the microbe-free work environment necessary for cell culture propagation, and also may be used for the formulation of nonvolatile antineoplastic or chemotherapeutic drugs.[30]

1. <u>The Class II, Type A BSC</u> - An internal blower (Figure 3) draws sufficient room air through the front grille to maintain a minimum calculated or measured 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 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 remainder to the rear grille. Although there are variations among different cabinets, this split generally occurs about half-way between the front and rear grilles, and two to six inches above the work surface.

The air is then discharged through the rear plenum into the space between the supply and exhaust filters located at the top of the cabinet. Due to the relative size of these two filters, approximately 30% of the air passes through the exhaust HEPA filter and 70% recirculates through the supply HEPA filter back into the work zone. Most Class II, Type A cabinets have dampers to modulate this 30/70 division of airflow.

An unducted Class II Type A BSC is not to be used for work involving volatile or toxic chemicals. The buildup of chemical vapors in the cabinet (by recirculated air) and in the laboratory (from exhaust air) could create health and safety hazards (see Section IV).

It is possible to duct the exhaust from a Type A cabinet out of the building. However, it must be done in a manner that does not alter the balance of the cabinet exhaust system, and thereby disturbing the internal cabinet air flow. The typical method of ducting a Type A cabinet is to use a "thimble",[13] or canopy hood, which maintains a small opening (usually 1 inch) around the cabinet exhaust filter housing (Figure 4). The volume of the exhaust must be sufficient to maintain the flow of room air into the space between the thimble unit and the filter housing.[a] The thimble must be removable or be designed to allow for operational testing of the cabinet (see Section VI). The performance of a cabinet with this exhaust configuration can be affected by fluctuations in the building exhaust system.

"Hard-ducting" (i.e., direct connection) of Class II Type A cabinets to the building exhaust system is not recommended unless a dedicated exhaust fan system with a dynamic flow balancing mechanism is provided. The building exhaust system must be precisely matched to the airflow from the cabinet in both volume and static pressure. However, fluctuations in air volume and pressure that are common to all building exhaust systems make it difficult to match the airflow requirements of the cabinet. A competent in-house maintenance and engineering staff is required to achieve this.

2. <u>The Class II, Type B1 BSC</u> - Some biomedical research requires the use of small quantities of certain hazardous chemicals, such as carcinogens. The powdered form of these carcinogens should be weighed or manipulated in a chemical fume hood or a static-air glove box equipped with a double-door airlock.. Carcinogens used in cell culture or microbial systems require both biological and chemical containment.[19]

The Class II, Type B cabinet originated with the National Cancer Institute (NCI)-designed Type 2 (later called Type B) biological safety cabinet (Figure 5A), which was designed for manipulations of minute quantities of these hazardous chemicals with *in vitro* biological systems. The National Sanitation Foundation (NSF) Standard 49 definition of Type B1 cabinets[24] includes this classic NCI design Type B, as well as cabinets

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[a] Contact manufacturers for any additional specifications.

without supply HEPA filters located immediately below the work surface (Figure 5B), and/or those with exhaust/recirculation downflow splits other than 70/30%.

The cabinet supply blowers draw room air (plus a portion of the cabinet's recirculated air) through the front grille and then through the supply HEPA filters located immediately below the work surface. This particulate-free air flows upward through a plenum at each side of the cabinet and then downward to the work area through a back-pressure plate. In some cabinets there is an additional supply HEPA filter to remove particulates that may be generated by the blower/motor system.

Room air is drawn through the face opening of the cabinet at a minimum inflow velocity of 100 lfpm. As with the Type A cabinet, there is a split in the down-flowing air stream just above the work surface. In the Type B cabinet, approximately 70 percent of the downflow air exits through the rear grille, passes through the exhaust HEPA filter, and is discharged from the building. The remaining 30 percent of the downflow air is drawn through the front grille. Since the air which flows to the rear grille is discharged into the exhaust system, activities that may generate hazardous chemical vapors or particulates should be conducted towards the rear of the cabinet.[27]

Type B1 cabinets must be hard-ducted, preferably to a dedicated exhaust system, or to a properly-designed laboratory building exhaust. As indicated earlier, blowers on laboratory exhaust systems should be located at the terminal end of the duct work. A failure in the building exhaust system may not be apparent to the user, as the supply blowers in the cabinet will continue to operate. A pressure-independent monitor should be installed to sound an alarm and shut off the BSC supply fan, should failure in exhaust air flow occur. Since this feature is not supplied by all cabinet manufacturers, it is prudent to install a sensor in the exhaust system as necessary. To maintain critical operations, laboratories using Type B BSCs should connect the exhaust blower to the emergency power supply.

3. The Class II, Type B2 BSC - This BSC is a total-exhaust cabinet; no air is recirculated within it (Figure 6). 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 in room air or outside air at the top of the cabinet, passes it through a HEPA filter and down into the work area of the cabinet. The building or cabinet 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) prior to discharge to the outside. Exhausting as much as 1200 cubic feet per minute of conditioned room air makes this cabinet expensive to operate.

Should the building or cabinet exhaust fail, the cabinet will be pressurized, resulting in a flow of air from the work area back into the laboratory. Cabinets built since the early 1980's usually have an interlock system (installed by the manufacturer) to prevent the supply blower from operating whenever the exhaust flow is insufficient; systems can be retrofitted if necessary. Exhaust air movement should be monitored by a pressure-independent device.

4. The Class II, Type B3 BSC - This biological safety cabinet (Figure 7) is an exhausted Type A cabinet having a minimum inward airflow of 100 lfpm. All positive pressure contaminated plenums within the cabinet are surrounded by a negative air pressure plenum. Thus, leakage from a contaminated plenum will be into the cabinet and not into the environment.

5. Special applications - Class II BSCs can be modified to accommodate special tasks. For example, the front sash can be modified by the manufacturer to accommodate the eye pieces of a microscope, or the work surface can be designed to accept a carboy, a centrifuge, or other equipment that requires

containment. A rigid plate with arm holes can be added if needed. Good cabinet design, microbiological aerosol tracer testing of the modification, and appropriate certification (see Section VII) are required to ensure that the basic systems operate properly after modification. Maximum containment potential is achieved only through strict adherence to proper practices and procedures (see Section V).

The Class III BSC

The Class III biological safety cabinet (Figure 8) was designed for work with microbiological agents assigned to biosafety level 4, and provides maximum protection to the environment and the worker. It is a gas-tight ($1 x 10^{-5}$ cc/sec leak rate) enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank (that is accessible through the cabinet floor) or double-door pass-through box (such as an autoclave) that can be decontaminated between uses. Reversing that process allows for safe removal of materials from the Class III biosafety cabinet. Both supply and exhaust air are HEPA filtered. Exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge to the outdoors. Airflow is maintained by a dedicated independent exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure (usually about 0.5 inches of water gauge).

Arm-length, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow for manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials. The trade-off is clearly on the side of maximizing personal safety. Depending on the design of the cabinet, the supply HEPA filter provides particulate-free, albeit somewhat turbulent, airflow within the work environment.

Several Class III cabinets can be joined together in a "line" to provide a larger work area. Such cabinet lines are custom-built; the equipment installed within the cabinet line (e.g., refrigerators, small elevators, shelves to hold small animal cage racks, microscopes, centrifuges, incubators, etc.) is generally custom-built as well. Furthermore, Class III cabinets are usually only installed in maximum containment laboratories that have controlled access and require special ventilation or other support systems (such as steam for autoclaves). The reader should consult more definitive literature on these systems.[16,21,23]

Horizontal Laminar Flow "Clean Bench"

Horizontal laminar flow “clean benches” (Figure 9A) are not BSCs. They 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. These benches should never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials. The worker can be exposed to materials (including proteinaceous antigens) being manipulated on the clean bench, which may cause hypersensitivity. Horizontal air flow “clean benches” should never be used as a substitute for a biological safety cabinet.

Vertical Laminar Flow "Clean Bench"

Vertical laminar flow clean benches (Figure 9B) also are not BSCs. They may be useful, for example, in hospital pharmacies when a clean area is needed for preparation of intravenous drugs. While these units generally have a sash, the air is usually discharged into the room under the sash, resulting in the same potential problems as the horizontal laminar flow clean benches.

SECTION IV

Laboratory Hazards and Risk Assessment

Primary containment is an important strategy to minimize exposure to the many chemical, radiological, and biological hazards encountered in the laboratory. An overview is provided in Table 2 of the various classes of BSCs, the level of protection afforded by each and the appropriate risk assessment considerations. Microbiological risks are assigned to biosafety levels 1 through 4 and are addressed in depth in *BMBL*.[6] BSCs in which chemical and radiological materials are used require design modifications in the cabinet or building exhaust system to include charcoal filters, since HEPA filters do not retain agents which vaporize or sublimate.

Chemicals in BSCs

Work with infectious microorganisms often requires the use of various chemical compounds, and many commonly used chemicals vaporize easily. Therefore, evaluation of the inherent hazards of the chemicals must be part of the risk assessment when selecting a BSC. In order to determine the greatest chemical concentration which might be entrained in the air stream following an accident or spill, it is necessary to evaluate the quantities to be used. Mathematical models are available to assist in these determinations.[27] The Threshold Limit Values for Chemical Substances[1] also will provide information on the risk of personnel exposure. As detailed in Section III, volatile or toxic chemicals should not be used in Class II, Type A cabinets since vapor buildup inside the cabinet presents a fire hazard.

The electrical systems of Class II cabinets are not spark-proof, so a chemical concentration that would approach the lower explosive limits of the compound is to be prohibited. Furthermore, since Class II, Type A cabinets return chemical vapors to the cabinet work space and the room, they may expose the operator and other room occupants to toxic chemical vapors.

A chemical fume hood, which is designed for work with volatile chemicals, should be used *in lieu* of a BSC. Chemical fume hoods are connected to an independent exhaust system and operate with single-pass air ducted directly outside the building. They also are used when manipulating chemical carcinogens.[19] Class I and Class II, Type B2 biological safety cabinets which are exhausted to the outdoors can be used when manipulating small quantities of volatile chemicals required in microbiological studies. The Class II, Type B1 cabinet also may be used with minute or tracer quantities of nonvolatile chemicals.[24]

Caution should be exercised in the use of Class II, Type B3 (ducted Type A) cabinets for work involving volatile toxic chemicals, because a change in the air balance between the cabinet and building exhaust may result in release of chemical vapors to the laboratory. The thimble exhaust connection helps minimize this problem. If minute quantities of volatile toxic chemicals are to be used in the Class II, Type B3 cabinet, then the building exhaust system must be monitored and preferably interlocked with the cabinet blower.

Many liquid chemicals, including nonvolatile antineoplastic and chemotherapeutic drugs and low-level radionuclides, can be safely handled inside a Class II, Type A cabinet.[30] Class II BSCs should not be used for labeling of biohazardous materials with radioactive iodine. For this work, ventilated containment devices are needed that may require both HEPA and charcoal filters in exhaust systems that are hard-ducted to the outside (Figure 10).

Many virology and cell culture laboratories use diluted preparations of chemical carcinogens[19,23] and other toxic substances. Prior to maintenance of the cabinet, careful evaluation must be made of potential problems associated with decontaminating the cabinet and the exhaust system. Air treatment systems, such as a charcoal filter in a bag-in/bag-out housing,[21] (Figure 13) may be required so that effluents meet applicable emission regulations.

Recommendations from the former Office of Research Safety of the National Cancer Institute[29] (which are still valid) stated that certain work involving the use of some chemical carcinogens (*in vitro* procedures) can be performed in a Class II cabinet which meets the following parameters: (1) that the exhaust air flow is sufficient to provide an inward flow of 100 lfpm at the face opening of the cabinet; (2) that contaminated air plenums under positive pressure are leak-tight; (3) that the cabinet air is discharged outdoors; NSF 49[24] currently recommends that Class II Type B cabinets have all biologically contaminated ducts and plenums under negative air pressure, or surrounded by negative pressure ducts and plenums.

Radiological Hazards in the BSC

As indicated above, volatile radionuclides such as I^{125} should not be used within Class II, Type A cabinets (see Table 2). When using nonvolatile radionuclides inside a BSC, the same hazards exist as when working with radioactive materials on the bench top. Work that has the potential for splatter or aerosolization can be done within the BSC. Monitoring for radioactivity must be done and BSCs be decontaminated as needed. When Appropriate, a vertical (not sloping) beta shield may be used inside the BSC to provide worker protection when appropriate.

Risk Assessment

The potential for untoward events must be evaluated to reduce or eliminate worker exposure to or release of infectious organisms. Agent summary statements detailed in *BMBL*[6] provide risk assessment data for microorganisms known to have caused laboratory-associated infections. Through the process of risk assessment, work procedures are evaluated for the potential to cause exposure to the microorganism.

The hierarchy of controls to prevent or minimize exposure to hazardous materials includes engineering controls, administrative and procedural controls, and work practices which may involve use of additional personal protective equipment. A properly operating BSC available is an effective engineering control (see Section VI), and requiring its use is an administrative control. Some suggested work practices and procedures associated with working safely in a BSC are detailed in the next section.

SECTION V

BSC Use by the Investigator: Work Practices and Procedures

Preparing for Work Within a Class II BSC

Preparing a written checklist of materials necessary for a particular activity and placing necessary materials in the BSC before beginning work serves to minimize the number of arm-movement disruptions across the fragile air barrier of the cabinet. The rapid movement of a worker's arms in a sweeping motion into and out of the cabinet will disrupt the air curtain and may compromise the partial barrier containment provided by the BSC. Moving arms in and out slowly, perpendicular to the face opening of the cabinet, will reduce this risk. Other personnel activities in the room (e.g., rapid movement, open/closing room doors, etc.) may also disrupt the cabinet air barrier.[5]

Laboratory coats should be worn buttoned over street clothing; latex gloves are worn to provide hand protection. A solid front, back-closing lab gown provides better protection of personal clothing than a traditional lab coat. Gloves should be pulled over the knitted wrists of the gown, rather than worn inside. Elasticized sleeves can also be worn to protect the investigator's wrists.

Before beginning work, the investigator should adjust the stool height so that his/her face is above the front opening. Manipulation of materials should be delayed for approximately one minute after placing the hands/arms inside the cabinet. This allows the cabinet to stabilize and to "air sweep" the hands and arms to remove surface microbial contaminants. When the user's arms rest flatly across the front grille, room air may flow directly into the work area, rather than being drawn through the front grille. Raising the arms slightly will alleviate this problem. The front grille must not be blocked with research notes, discarded plastic wrappers, pipetting devices, etc. All operations should be performed on the work surface at least four (4) inches from the inside edge of the the front grille.

BSC Use: Work Practices and Procedures

Closure of the drain valve under the work surface should be done prior to beginning work so that all contaminated materials are contained within the cabinet should a large spill occur.

Materials or equipment placed inside the cabinet may cause disruption to the airflow, resulting in turbulence, possible cross-contamination, and/or breach of containment. Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet. Only the materials and equipment required for the immediate work should be placed in the BSC.

BSCs are designed to be operated 24 hours per day, and some investigators find that continuous operation helps to control the laboratory's level of dust and other airborne particulates. Although energy conservation may suggest BSC operation only when needed, especially if the cabinet is not used routinely, room air balance is an overriding consideration. In some instances, room exhaust is balanced to include air discharged through ducted BSCs.

Cabinet blowers should be operated at least three to five minutes before beginning work to allow the cabinet to "purge". This purge will remove any particulates in the cabinet. The work surface, the interior walls (not including the supply filter diffuser), and the interior surface of the window should be wiped with 70% ethanol (EtOH), a 1:100 dilution of household bleach (i.e., 0.05% sodium hypochlorite), or other disinfectant as determined by the investigator to meet the requirements of the particular activity. When bleach is used, a second wiping with sterile water is needed to remove the residual chlorine, which may eventually corrode stainless steel surfaces. Wiping with non-sterile water may recontaminate cabinet surfaces, a critical issue when sterility is essential (e.g., maintenance of cell cultures).

Similarly, the surfaces of all materials and containers placed into the cabinet should be wiped with 70% EtOH to reduce the introduction of contaminants to the cabinet environment. This simple step will reduce introduction of mold spores and thereby minimize contamination of cultures. Further reduction of microbial load on materials to be placed or used in BSCs may be achieved by periodic decontamination of incubators and refrigerators.

Material Placement Inside the BSC

Plastic-backed absorbent toweling can be placed on the work surface (but not on the front or rear grille openings). This toweling facilitates routine cleanup and reduces splatter and aerosol formation[3] during an overt spill. It can be folded and placed in an autoclavable biohazard bag when work is completed.

All materials should be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet (Figure 11). Similarly, aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) should be placed toward the rear of the cabinet to take advantage of the air split described in Section III. Active work should flow from the clean to contaminated area across the work surface. Bulky items such as biohazard bags, discard pipette trays and suction collection flasks should be placed to one side of the interior of the cabinet.

Certain common practices interfere with the operation of the BSC. The autoclavable biohazard collection bag should not be taped to the outside of the cabinet. Upright pipette collection containers should not be used in BSCs nor placed on the floor outside the cabinet. The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. Only horizontal pipette discard trays containing an appropriate chemical disinfectant should be used within the cabinet. Furthermore, potentially contaminated materials should not be brought out of the cabinet until they have been surface decontaminated. Alternatively, contaminated materials can be placed into a closable container for transfer to an incubator, autoclave or for other decontamination treatment.

Operations Within a Class II BSC

Laboratory Hazards

Many procedures conducted in BSCs may create splatter or aerosols. Good microbiological techniques should always be used when working in a biological safety cabinet. For example, techniques to reduce splatter and aerosol generation will minimize the potential for personnel exposure to infectious materials manipulated within the cabinet. Class II cabinets are designed so that horizontally nebulized spores introduced into the cabinet will be captured by the downward flowing cabinet air within fourteen inches[24] of travel. Therefore, as a general rule of thumb, keeping clean materials at least one foot away from aerosol-generating activities will minimize the potential for cross-contamination.

The work flow should be from "clean to contaminated (dirty)" (see Figure 11). Materials and supplies should be placed in such a way as to limit the movement of "dirty" items over "clean" ones.

Several measures can be taken to reduce the chance for cross-contamination when working in a BSC. Opened tubes or bottles should not be held in a vertical position. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impaction of downward air. Bottle or tube caps should not be placed on the toweling. Items should be recapped or covered as soon as possible.

Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current which prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence which disrupts the pattern of HEPA-filtered air supplied to the work surface. When deemed absolutely necessary, touch-plate microburners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops can also be used.

Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter (see Figure 12). This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to kill the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste.

Investigators must determine the appropriate method of decontaminating materials that will be removed from the BSC at the conclusion of the work. When chemical means are appropriate, suitable liquid disinfectant should be placed into the discard pan before work begins. Items should be introduced into the pan with minimum splatter, and allowed appropriate contact time as per manufacturer's instructions. Alternatively, liquids can be autoclaved prior to disposal. Contaminated items should be placed into a biohazard bag or discard tray inside the BSC. Water should be added to the bag or tray prior to autoclaving.

When a steam autoclave is to be used, contaminated materials should be placed into a biohazard bag or discard pan containing enough water to ensure steam generation during the autoclave cycle. The bag should be taped shut or the discard pan should be covered in the BSC prior to removal to the autoclave. The bag should be transported and autoclaved in a leakproof tray or pan. It is a prudent practice to decontaminate the exterior surface of bags and pans just prior to removal from the cabinet.

BSC Use: Work Practices and Procedures

Decontamination

Surface Decontamination

All containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. At the end of the work day, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back, and the interior of the glass. If necessary, the cabinet should also be monitored for radioactivity and decontaminated when necessary. Investigators should remove their gloves and gowns in a manner to prevent contamination of unprotected skin and aerosol generation and wash their hands as the final step in safe microbiological practices.

Small spills within the BSC can be handled immediately by removing the contaminated absorbent paper toweling and placing it into the biohazard bag. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately wiped with a towel dampened with decontaminating solution. Gloves should be changed after the work surface is decontaminated and before placing clean absorbent toweling in the cabinet. Hands should be washed whenever gloves are changed or removed.

Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination. All items within the cabinet should be surface decontaminated and removed. Decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan.

Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. Manufacturer's directions should be followed. The spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag. The drain pan should be emptied into a collection vessel containing disinfectant. A flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. This procedure serves to minimize aerosol generation. The drain pan should be flushed with water and the drain tube removed.

Should the spilled liquid contain radioactive material, a similar procedure can be followed. Radiation safety personnel should be contacted for specific instructions.

Gas Decontamination

BSCs that have been used for work involving infectious materials must be decontaminated before HEPA filters are changed or internal repair work is done.[4,9-12] Before a BSC is relocated, a risk assessment which considers the agents manipulated within the BSC must be done to determine the need for decontamination. The most common decontamination method uses formaldehyde gas, although more recently hydrogen peroxide vapor[10] has been used successfully. This environmentally benign vapor is useful in decontaminating HEPA filters, isolation chambers and centrifuge enclosures.[11]

SECTION VI

Facility and Engineering Requirements

Secondary Barriers

Whereas biological safety cabinets are considered to be the primary safety barrier for manipulation of infectious materials, the laboratory room itself is considered to be the secondary safety barrier.[22] Inward directional air flow is established[2] by exhausting a greater volume of air than is supplied to a given laboratory and by drawing makeup air from the adjacent space. This is optional at biosafety level 2 but must be maintained at BSL-3.[6,28] The air balance for the entire facility should be established and maintained to ensure that air flow is from areas of least- to greater contamination.

Building Exhaust

At BSL-3 and BSL-4, exhaust laboratory air must be directly exhausted since it is considered potentially contaminated. This concept is referred to as a dedicated single-pass exhaust system. The exhausted room air can be HEPA-filtered when a high level of aerosol containment is needed, which is always true at BSL-4 and is optional at BSL-3. When the building exhaust system is used to vent a ducted BSC, the system must have a sufficient capacity to maintain the exhaust flow if changes in the static pressure within the system should occur. Otherwise, each cabinet must have a dedicated exhaust system.

The room exhaust system should be sized to handle both the room and all containment devices vented through the system. Adequate supply air must be provided to ensure appropriate function of the exhaust system. The facility engineer should be consulted before locating a new cabinet requiring connection to the building exhaust system. Right angle bends, long horizontal runs, and transitional connectors within the systems will add to the demand on the exhaust fan. The building exhaust air should be discharged away from supply air intakes, to prevent entrainment of exhausted laboratory air back into the building air supply system.

Facility and Engineering Requirements

Utility Services

Utility services needed within a BSC must be planned carefully. Protection of vacuum systems must be addressed (Fig. 12). Electrical outlets inside the cabinet must be protected by ground fault circuit interrupters and should be supplied by an independent circuit. When propane gas is provided, a clearly marked emergency gas shut-off valve outside the cabinet must be installed for fire safety. Consider providing a timed shutoff valve for the gas service. All nonelectrical utility services should have exposed, accessible shut-off valves. Compressed air should not be provided because of the potential for aerosol generation in the event a pressurized vessel fails.

Ultraviolet Lamps

Ultraviolet (UV) lamps are not required in BSCs. If installed, UV lamps must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the ultraviolet light. The lamps should be checked periodically with a meter to ensure that the appropriate intensity of UV light is being emitted. UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer.

BSC Placement

Biological safety cabinets were developed (see Section I) as work stations to provide personnel, product and environmental protection during the manipulation of infectious microorganisms. Certain considerations must be met to ensure maximum effectiveness of these primary barriers. Whenever possible, an adequate clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance, and to ensure that the air return to the laboratory is not hindered. A 12- to 14-inch clearance above the cabinet may be required to provide for accurate air velocity measurement across the exhaust filter surface[14,15] and for exhaust filter changes. When the BSC is hard-ducted or connected by a thimble unit to the ventilation system, adequate space must be provided so that the configuration of the duct work will not interfere with air flow. The thimble unit must provide access to the exhaust filter for testing of the HEPA filter.

The ideal location for the biological safety cabinet is remote from the entry (i.e., the rear of the laboratory away from traffic), since people walking parallel to the face of a BSC can disrupt the air curtain.[5,23,25] The air curtain created at the front of the cabinet is quite fragile, amounting to a nominal inward and downward velocity of 1 mph. Open windows, air supply registers, or laboratory equipment that creates air movement (e.g., centrifuges, vacuum pumps) should not be located near the BSC. Similarly, chemical fume hoods must not be located close to BSCs.

HEPA Filters

HEPA filters, whether part of a building exhaust system or part of a cabinet, will require replacement when they become loaded to the extent that sufficient air flow can no longer be maintained. Filters must be decontaminated before removal. To contain the formaldehyde gas typically used for microbiological decontamination, exhaust systems containing HEPA filters require airtight dampers to be installed on both the inlet and discharge side of the filter housing. This ensures containment of the gas inside the filter housing during decontamination. Access panel ports in the filter housing also allow for performance testing of the HEPA filter (see Section VII).

A bag-in/bag-out (BIBO) filter assembly[9,21] (Figure 13) can be used in situations where HEPA filtration is necessary for operations involving biohazardous materials and hazardous or toxic chemicals. The BIBO system is used when it is not possible to decontaminate the HEPA filters with formaldehyde gas, or when hazardous toxic chemicals have been used in the BSC, but provides protection against exposure for the maintenance personnel and the environment Note, however, that this requirement must be identified at the time of purchase and installation; a BIBO assembly cannot be added to a cabinet after-the-fact.

SECTION VII

Certification of Biological Safety Cabinets

Development of Containment Standards

The evolution of containment equipment for varied research and diagnostic applications created the need for consistency in construction, certification and performance. A Federal standard was developed[b] to establish classes of air cleanliness and methods for monitoring clean work stations and clean rooms where HEPA filters are used to control airborne particulates.

The first "standard" to be developed specifically for BSCs[17] served as a Federal procurement specification for the NIH Class II, Type 1 (now called Type A) biological safety cabinet, which had a fixed or hinged front window or a vertical sliding sash, vertical downward laminar airflow and HEPA-filtered air supply and exhaust. This guideline specified design criteria and defined prototype tests for microbiological aerosol challenge, velocity profiles, and leak testing of the HEPA filters. A similar procurement specification was generated[20] when the Class II Type 2 (now called Type B1) cabinet was developed.

The National Sanitation Foundation (NSF International) Standard No. 49 for Class II (Laminar Flow) Biohazard Cabinetry[24] was first published in 1976, providing the first independent standard for design, manufacture and testing the BSCs. This standard "replaced" the NIH specifications which were being used

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[b] Federal Standard No. 209B, Clean Room and Work Station Requirements"[7] has evolved into Federal Standard No. 209E, Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones".[8] This standard does not apply to BSCs and should not be considered a basis for their performance or integrity certification. However, the methodology of 209E can be used to quantify the particulate count within the work area of a BSC. 209E defines how to classify a cleanroom/clean zone. Performance tests and procedures needed to achieve a specified cleanliness classification are outlined by the Institute of Environmental Sciences - Testing Clean Rooms (IES-RP-CC-006-84-T) and Laminar Flow Clean Air Devices

by other institutions and organizations purchasing BSCs. NSF Standard 49 incorporates specifications regarding design, materials and construction. This Standard for biological safety cabinets establishes performance criteria and provides the minimum requirements that are accepted in the United States. Cabinets which meet the standard and are certified by the NSF bear an NSF 49 Seal.

Standard No. 49 pertains to all models of Class II cabinets (Type A, B1, B2, and B3) and lists a series of specifications regarding:

- design/construction,
- performance,
- installation recommendations,
- recommended microbiological decontamination procedure, and
- references and specifications pertinent to Class II Biohazard Cabinetry.

While the NSF standard does not cover field testing of BSCs, it is common for many of its test methods and parameters to be applied in the field, and these are included in Annex "F" of the standard. Most recently revised in 1992 (with a new revision due in 2000),[24] this Standard is reviewed periodically by a steering committee to ensure that it remains consistent with developing technologies.[c]

The operational integrity of a new BSC must be validated before it is put into service or after a cabinet has been repaired or relocated. Relocating a BSC may break the HEPA filter seals or otherwise damage the filters or the cabinet. Each BSC should be tested and certified at least annually to ensure continued proper operation.

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[c] The standard can be ordered from NSF for a nominal charge at NSF International, 3475 Plymouth Road, P.O. Box 130140, Ann Arbor, Michigan, 48113-0140 USA. Telephone 313-769-8010; Fax 313-769-0109; Telex 753215 NSF INTL

Certification of Biosafety Cabinets

On-site testing following the recommendations for field testing (NSF Standard 49) must be performed by experienced, qualified personnel. Some basic information is included here to assist in understanding the frequency and kinds of tests to be performed. In 1993, NSF began a program for accreditation of certifiers based on written and practical examinations. Education and training programs for persons seeking accreditation as qualified to perform all field certification tests are offered through the Eagleson Institute, Sanford, ME; the Harvard School of Public Health, Cambridge, MA; NuAire Inc., Plymouth, MN; Forma Scientific Inc., Marietta, OH; and Lab Conco Corporation, Kansas City, MO. Other training, education and certification programs may be developed in the future. Selecting competent individuals to perform testing and certification is important, and it is suggested that the institutional biosafety officer be consulted in identifying companies qualified to conduct the necessary field performance tests.

It is strongly recommended that whenever possible accredited field certifiers be used to test and certify BSCs If in-house personnel are preforming the certifications, then these individuals should become accredited. The importance of proper certification cannot be emphasized enough, since persons who manipulate infectious microorganisms are at increased risk of acquiring an occupational illness when their BSCs are functioning improperly.

The annual tests applicable to each of the three classes of biological safety cabinets are listed in Table 3. Table 4 indicates where to find information regarding the conduct of selected tests. BSCs perform consistently well when proper annual certification procedures are followed; cabinet or filter failures tend to occur infrequently.

Performance Testing BSCs in the Field

BSCs are the primary containment device that protect the worker, product and environment from exposure to microbiological agents. Their operation as specified by Standard No. 49 needs to be verified at the time of installation and annually thereafter. The purpose and acceptance level of the performance tests (Table 3) are to ensure the balance of inflow and exhaust air, the distribution of air onto the work surface, and the integrity of the cabinet. Other tests check electrical and physical features of the BSC.

A. Downflow Velocity: This test is performed to measure the velocity of air moving through the cabinet workspace, and is to be performed on all biosafety cabinets.

B. Inflow Velocity Test: This test is performed to determine the calculated or directly measured velocity through the work access opening, to verify the nominal set point average inflow velocity and to calculate the exhaust airflow volume rate.

C. Airflow Smoke Patterns Tests: This test is performed to determine if the airflow along the entire perimeter of the work access opening is inward, if airflow within the work area is downward with no dead spots or refluxing, if ambient air passes onto or over the work surface, and if there is refluxing to the outside at the window wiper gasket and side seals. The smoke test is an indicator of airflow direction, not velocity.

D. HEPA Filter Leak Test: This test is performed to determine the integrity of supply and exhaust HEPA filters, filter housing, and filter mounting frames while the cabinet is operated at the nominal set point velocities. An aerosol in the form of generated particulates of dioctylphthalate (DOP) or an accepted alternative (e.g., food grade corn oil, di(2-ethylhexyl), sebecate, polyethylene glycol, and medical grade light mineral oil) is required for leak-testing HEPA filters and their seals. Although DOP has been identified as a potential carcinogen, competent service personnel are trained to use this chemical in a safe manner. The aerosol is generated on the intake side of the filter, and particles passing through the filter or around the seal are measured with a photometer on the discharge side. This test is suitable for ascertaining the integrity of all HEPA filters.

E. Cabinet Leak Test: The pressure holding test is performed to determine if exterior surfaces of all plenums, welds, gaskets, and plenum penetrations or seals are free of leaks. It need only be performed just prior to initial installation when the BSC is in a free-standing position (all four sides are easily accessible) in the room in which it will be used, after a cabinet has been relocated to a new location, and again after removal of access panels to plenums for repairs or a filter change. This test may also be performed on fully installed cabinets. "Cabinet integrity can also be checked using the bubble test ".

F. Electrical Leakage and Ground Circuit Resistance and Polarity Tests: These safety tests are performed to determine if a potential shock hazard exists by measuring the electrical leakage, polarity, ground fault interrupter function, and ground circuit resistance to the cabinet connection. They may be performed by an electrical technician other than the field certification personnel at the same time the other field certification tests are conducted. The polarity of electrical outlets are checked (see Table 3, E). The ground fault circuit interrupter should trip when approximately 5 milliamperes (ma) is applied.

G. Lighting Intensity Test: This test is performed to measure the light intensity on the work surface of the cabinet as an aid in minimizing cabinet operator's fatigue.

H. Vibration Test: This test is performed to determine the amount of vibration in an operating cabinet as a guide to satisfactory mechanical performance, as an aid in minimizing cabinet operator's fatigue, and to prevent damage to delicate tissue culture specimens.

I. Noise Level Test: This test is performed to measure the noise levels produced by the cabinets, as a guide to satisfactory mechanical performance and an aid in minimizing cabinet operator's fatigue.

J. UV Lamp Test: A few BSCs have UV lamps. When used, they must be tested periodically to ensure that their energy output is sufficient to kill microorganisms. After having been turned off and allowed to cool, the surface on the bulb should be cleaned with 70% ethanol prior to performing this test.

Certification of Biosafety Cabinets

Five minutes after the lamp has been turned on, the sensor of the UV meter is placed in the center of the work surface. The radiation output should not be less than 40 microwatts per square centimeter at 254 nanometers (nm).

Finally, accurate test results can only be assured when the testing equipment is properly maintained and calibrated. It is appropriate to request the calibration information for the test equipment being used by the certifier.

Table 1. Selection of a Safety Cabinet Through Risk Assessment

Biological Risk Assessed	Protection Provided			BSC Class
	Personnel	Product	Environmental	
BSL 1-3	YES	NO	YES	I
BSL 1-3	YES	YES	YES	II (A, B1, B2, B3)
BSL 4	YES	YES	YES	III B1, B2

Table 2. Comparison of Biosafety Cabinet Characteristics

BSC Class	Face Velocity (fpm)	Airflow Pattern	Applications	
			Nonvolatile Toxic Chemicals and Radionuclides	Volatile Toxic Chemicals and Radionuclides
I	75	In at front; exhausted through HEPA to the outside or into the room through HEPA (See Figure 2)	YES	YES[1]
II, A	75	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to outside through a thimble unit	YES (minute amounts)	NO
II, B1	100	Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter	YES	YES (minute amounts)[2]
II, B2	100	No recirculation; total exhaust to the outside through hard-duct and a HEPA filter	YES	YES (small amounts)
II, B3	100	Same as II,A, but plenums are under negative pressure to room; exhaust air is thimble-ducted to the outside through a HEPA filter	YES	YES (minute amounts)[2]
III	N/A	Supply air inlets and hard-duct exhausted to outside through two HEPA filters in series	YES	YES (small amounts)

1. Installation may require a special duct to the outside, an in-line charcoal filter, and a spark proof (explosion proof) motor and other electrical components in the cabinet. Discharge of a Class I cabinet into a room should not occur if volatile chemicals are used.

2. In no instance should the chemical concentration approach the lower explosion limits of the compound.

Table 3. Operational Tests to be Applied to the Three Classes of Biological Safety Cabinets

TESTS PERFORMED FOR	BIOSAFETY CABINET CLASS I	CLASS II	CLASS III
Primary Containment			
Cabinet Integrity	N/A	A	A
HEPA Filter Leak	Req	Req	Req
Downflow Velocity Profile	N/A	Req	N/A
Face Velocity	Req	Req	N/A
Negative Pressure/Ventilation Rate	B	N/A	Req
Airflow Smoke Patterns	Req	Req	E/F
Alarms and Interlocks	C,D	C,D	Req
Electrical Safety			
Electrical Leakage, etc.	D,E	D,E	D,E
Ground Fault Interrupter	D	D	D
Other			
Lighting Intensity	E	E	E
UV Intensity	C,E	C,E	C,E
Noise Level	E	E	E
Vibration	E	E	E

Req Required during certification.
A Required for proper certification if the cabinet is new, has been moved or panels have been removed for maintenance.
B If used with gloves.
C If present.
D Encouraged for electrical safety.
E Optional, at the discretion of the user.
F Used to determine air distribution within cabinet for clean to dirty procedures.
N/A Not applicable.

Table 4. References for Applicable Containment Tests

TEST	CABINET TYPE BY CLASS I	II	III
HEPA Filter Leak	(F,IID)[1]	(F,IID)	(F,IID)
Airflow Smoke Pattern	No smoke shall reflux out of BSC once drawn in.	(F,IIC)	N/A
Cabinet Integrity	N/A	(F,IIE)	N/A
Face Velocity Open Front	75-125 lfpm (F,IIB,3b)	75 lfpm - type A; 100 lfpm - type B (F,IIB)	N/A
Face Velocity Glove Ports/No Gloves	150 lfpm (F,IIB,3b(1)(C))	N/A	100 lfpm
Water Gauge Pressure Glove Ports & Gloves	N/A	N/A	-0.5" [p.145][2]
Velocity Profile	N/A	(F,IIA)	N/A

1. Parenthetical references are to the NSF Standard 49;[23] letters and numerals indicate specific sections and subsections.

2. Bracketed reference ([]) is to the Laboratory Safety Monograph;[22] page numbers are indicated.

FIGURES

Figure 1. HEPA filters are typically constructed of paper-thin sheets of borosilicate medium, pleated to increase surface area, and affixed to a frame. Aluminum separators are often added for stability.

Figures

Figure 2. The Class I BSC. A. front opening, B. sash, C. exhaust HEPA filter, D. exhaust plenum.

Figure 3. The Class II, Type A BSC. A. front opening, B. sash, C. exhaust HEPA filter, D. rear plenum, E. supply HEPA filter, F. blower.

Figures

Figure 4. Typical thimble unit for ducting a Class II, Type A BSC. A. balancing damper, B. flexible connector to exhaust system, C. cabinet exhaust HEPA filter housing, D. thimble unit, E. BSC. Note: There is a 1" gap between the thimble unit (D) and the exhaust filter housing (C), through which room air is exhausted. Care must be taken to match thimble design with the exhaust airflow characteristics of the cabinet.

Figure 5A. The Class II, Type B1 BSC (classic design). A. front opening, B.sash, C. exhaust HEPA filter, primary supply HEPA filter. D. supply HEPA filter, E. negative pressure exhaust plenum, F. blower, G. additional HEPA filter for supply air., Note: The cabinet exhaust needs to be connected to the building exhaust system as specified by the manufacturer.

Figure 5B. The Class II, Type B1 BSC (bench top design). A. front opening, B. sash, C. exhaust HEPA filter, D. supply plenum, E. supply HEPA filter, F. blower, G. negative pressure exhaust plenum. Note: The cabinet exhaust needs to be connected to the building exhaust system as specified by the manufacturer.

Figures

Figure 6. The Class II, Type B2 BSC. A. front opening, B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. negative pressure exhaust plenum, F. filter screen, supply blower.
G. "Supply diffuser". The cabinet exhaust must be connected to an exhaust system capable of providing the static pressure required to operate the cabinet.

Figure 7. The tabletop model of a Class II, Type B3 BSC. A. front opening, B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. positive pressure plenum, F. negative pressure plenum.
Note: The cabinet exhaust needs to be connected to the building exhaust system as specified by the manufacturer.

Figures

Figure 8. The Class III BSC. A. glove ports with O-ring for attaching arm-length gloves to cabinet, B. fixed window, 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 exhaust system.

Figure 9A. The horizontal laminar flow "clean bench". A. front opening, B. supply grille, C. supply HEPA filter, D. supply plenum, E. blower, F. grille.

Figure 9B. The vertical laminar flow "clean bench". A. front opening, B. sash, C. supply HEPA filter, D. blower.

Figures

Figure 10. A modified containment cabinet or Class I BSC can be used for labelling infectious microorganisms with I^{125}. A. arm holes, B. Lexan^{R} hinged doors, C. exhaust charcoal filter, D. exhaust HEPA filter, E. filter housing with required connection to building exhaust (see also Figure 13).

Figures

Figure 11. A typical layout for working "clean to dirty" within a Class II BSC. 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.

Figure 12. One method to protect a house vacuum system during aspiration of infectious fluids. The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask serves as a fluid overflow collection vessel. A glass sparger in flask B minimizes splatter. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.

Figures

Figure 13. A bag-in-bag-out filter enclosure allows for the removal of the contaminated filter without worker exposure. A. filters, B. bags, C. safety straps, D. cinching straps, E. shock cord located in the mouth of the PVC bag restricts the bag around the second rib of the housing lip.

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[d] Note: as we go to press, this publication is out of print or unavailable. Research may be necessary to review the text.