OCCUPATIONAL AND ENVIRONMENTAL SAFETY

Biological Safety Cabinets – Work Practices and Procedures

This training is for information only.

Overview

OCCUPATIONAL AND ENVIRONMENTAL SAFETY

Biological Safety Cabinets (BSCs) are an indispensable piece of safety equipment in the microbiological and biomedical laboratory; however, they only protect when they are used properly. The following training is excerpted from the CDC publication " Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets" which can be viewed online here.

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.

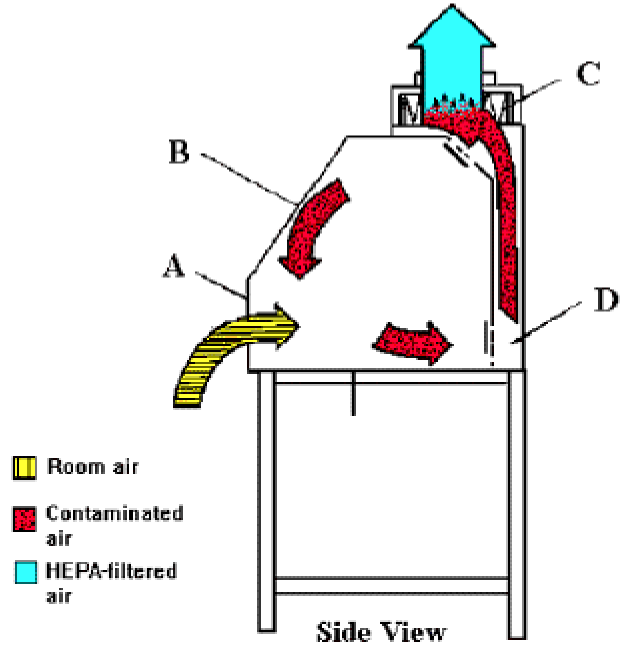
Class I BSC

The Class I BSC is essentially a biological version of the traditional laboratory fume hood (chemical hood), and 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. In the Class I BSC, unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward airflow as long as a minimum velocity of 75 linear feet per minute (lfpm) is maintained through the front opening. 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 can be 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.



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. 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 and provide necessary product protection.

The Class II (Types A1 (formerly Type A), A2 (formerly B3), B1, and B2) 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. In certain cases, a charcoal filter may be added to prevent release of toxic chemicals into the atmosphere.

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

Class II BSC

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 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

Class II, Type A1 BSC

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In a Class II , Type A1 Biosafety Cabinet, an internal blower draws room air through the front grille to maintain a minimum average inflow velocity of at least 75 Linear Feet Per Minute (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 A1 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.
It is possible to duct the exhaust from a Type A1 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 A1 cabinet is to use a "thimble" or canopy hood, which maintains a small opening (usually 1 inch) around the cabinet exhaust filter housing. 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. The thimble must be removable or be designed to allow for operational testing of the cabinet. 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 A1 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.

Class II, Type A1 BSC

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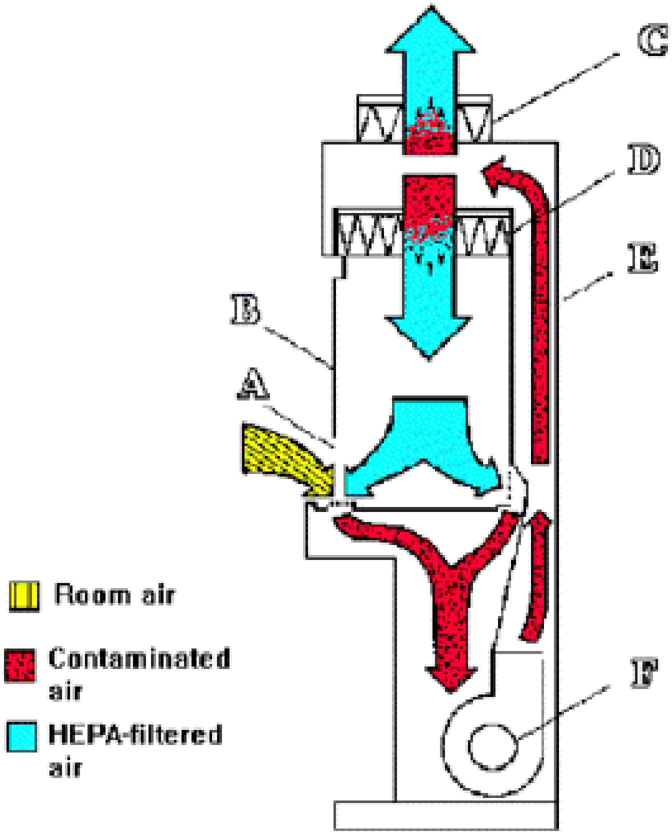

Side View

Class II, Type A2 BSC

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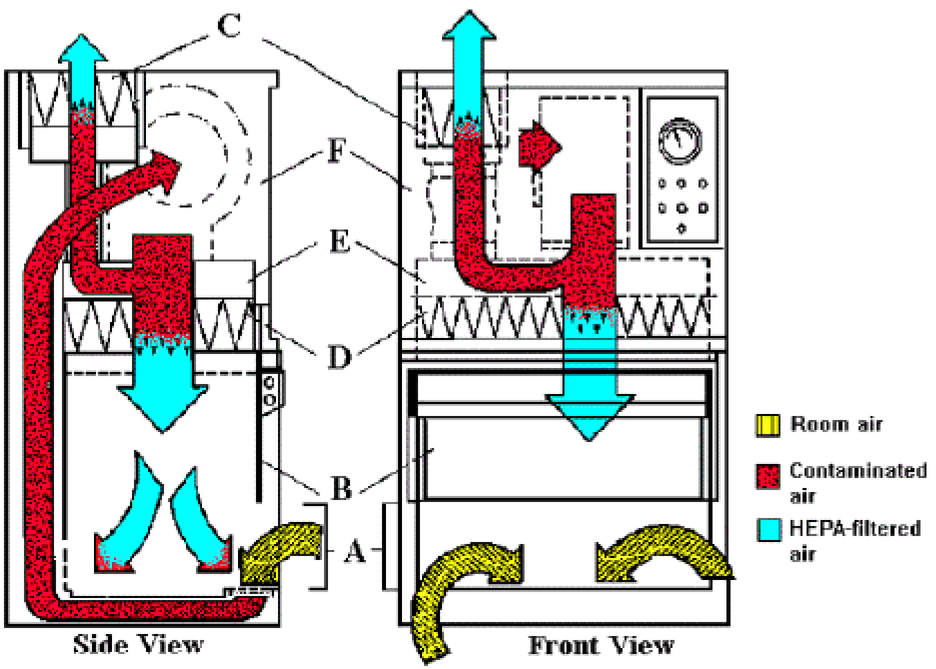
This biological safety cabinet (originally the Class II, Type B3) is an exhausted Type A1 cabinet having a minimum inward airflow of 100 lfpm. All positive pressure contaminated plenums within the cabinet are surrounded by a negative air pressure plenum. Thus, leakage from a contaminated plenum will be into the cabinet and not into the environment.



Class II, Type B1 BSC

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.

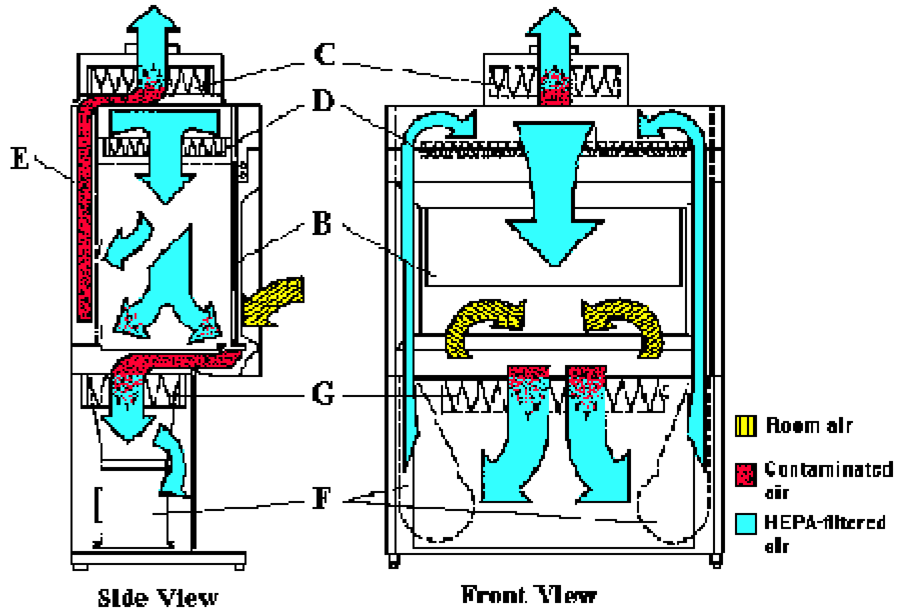
The Class II, Type B cabinet originated with the National Cancer Institute (NCI)-designed Type 2 (later called Type B) biological safety cabinet, which was designed for manipulations of minute quantities of these hazardous chemicals with in vitro biological systems. The National Sanitation Foundation (NSF) Standard 49 definition of Type B1 cabinets includes this classic NCI design Type B, as well as cabinets without supply HEPA filters located immediately below the work surface, 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.

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.

Class II, Type B1 BSC



Class II, Type B2 BSC

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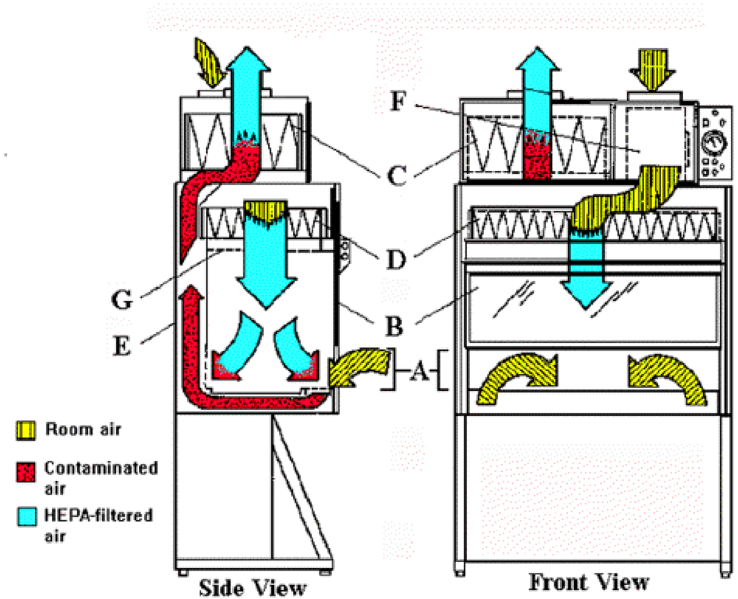
This BSC is a total-exhaust cabinet; no air is recirculated within it. This cabinet provides simultaneous primary biological and chemical containment.

Consideration must be given to the chemicals used in this type BSC, 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 fpm. 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.



Class III BSC

The Class III biological safety cabinet 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 (1x10-5 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).

Clean benches

Horizontal laminar flow "clean benches" 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" must never be used as a substitute for a biological safety cabinet.

Vertical laminar flow clean benches 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.

Preparing for work in a Class II BSC

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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.

Laboratory coats should be worn buttoned over street clothing; appropriate gloves are worn to provide hand protection. Note that 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 at least four "4" inches from the front grille on the work surface.

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 operate continuously and should be on 24 hours per day; some investigators find that continuous operation helps to control the laboratory's level of dust and other airborne particulates. If a BSC is operated intermittently, the BSC blowers should be started **at least** three to five minutes before beginning work to allow the cabinet to " purge". This purge will remove any particulates in the cabinet. To enhance the sterility of cabinets after they have been shut off, 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

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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 during an overt spill. lt then 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. 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. 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.

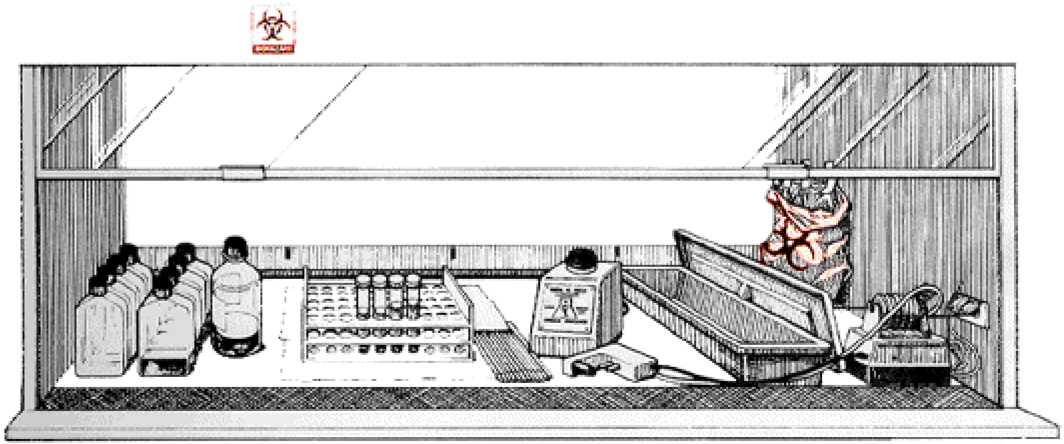
The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. Only horizontal pipette discard trays containing an appropriate chemical disinfectant should be used within 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.

Hazards in a BSC

Many common 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 11 cabinets are designed so that horizontally nebulized spores will be captured by the downward flowing cabinet air within fourteen inches 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 general workflow should be from "clean to contaminated (dirty)". Materials and supplies should be placed in such a way as to limit the movement of "dirty" items over "clean" ones.



Hazards in a BSC

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 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 appropriately 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.

Surface Decontamination

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All containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. At the end of the workday, 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 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. After ensuring that the drain valve is closed, 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 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. 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 has been used successfully. This environmentally benign vapor is useful in decontaminating HEPA filters, isolation chambers and centrifuge enclosures.

Ultraviolent (UV) Lights

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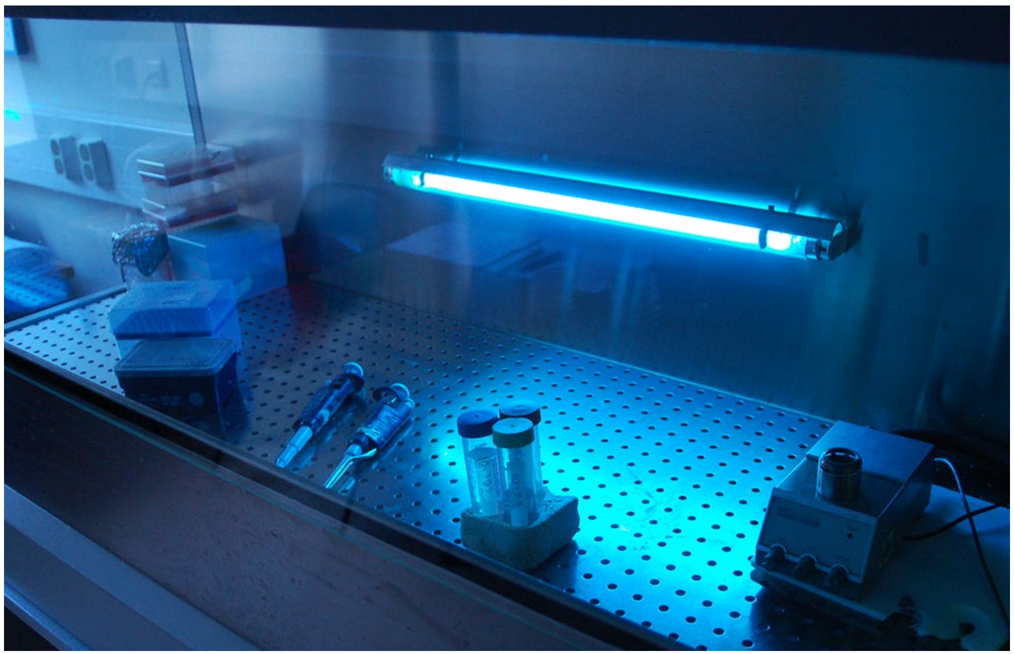

Ultraviolet (UV) lamps are not required in BSCs and are not recommended. Operated properly (i.e., left running continuously or properly purged prior to use) BSCs do not need UV lights.

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.

Review

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Biological safety cabinets were developed as workstations to provide personnel, product and environmental protection during the manipulation of infectious microorganisms. However, to provide adequate protection to personnel, certain considerations must be met, and practices be used to ensure maximum effectiveness of BSCs.

This overview is not meant to be definitive or all-encompassing. It is provided to clarify the expectations, functions and performance of Biosafety Cabinets - a critical primary barrier in the microbiological and biomedical laboratory. Further training is available by emailing the Biosafety Officer.

Lamarck is constantly reminded of his job security, for he is the only biologist that can read the biosafety cabinet's display.