

Chapter 16 Biosafety Cabinets and Other Laboratory Equipment

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Chemical Fume Hoods and Clean Benches

Some lab workers refer to biosafety cabinets as "hoods" or "laminar flow hoods". It is important to know the difference between a biosafety cabinet, a chemical fume hood and a clean bench. Biosafety cabinets are designed to protect the individual and the environment from biological agents, and to protect the research materials from contamination. Chemical fume hoods, however, are designed solely to protect the individual from exposure to chemicals and noxious gases. Since chemical fume hoods are not equipped with HEPA filters, they must not be used for work with biohazardous materials. Horizontal laminar flow hoods or "clean benches" are not acceptable for work with biological materials and any use must be approved by the Institutional Biosafety Committee. The air is HEPA filtered and directed across the bench top toward the user. Thus, it offers no protection to the user, only the product and therefore

has limited applications.

Biosafety Cabinets

Various laboratory procedures generate aerosols that may spread biohazardous material in the work area and pose a risk of infection to the worker. Biological safety cabinets (BSC) are used to prevent the escape of aerosols or droplets and to protect the research product from airborne contamination.

These devices are distinct from horizontal or vertical laminar flow "clean benches," which should never be used for handling biohazardous, toxic or sensitizing material.

Types of Biosafety Cabinets

Three major classes of biosafety cabinets are Class I, Class II, and Class III.

Class I biological safety cabinets are enclosures similar to chemical fume hoods, with an inward airflow through the front opening. The exhaust air from the biological safety cabinet is passed through a HEPA filter so that the equipment provides protection for the worker and the public. The product (research material) in the cabinet, however, is subject to contamination.

Class II biological safety cabinets are designed to protect the worker, the general public and the product. Class II cabinets are vertical laminar-flow cabinets with a partially open front. Airborne contaminants in the cabinet are prevented from escaping across this opening by a curtain of air formed by unfiltered air flowing from the room into the cabinet and HEPA filtered air supplied from an overhead grill down into the cabinet. A portion of the filtered air is used to maintain the air curtain, and the remainder passes down onto the work surface, and is drawn out through the grills at the back and front edges of the work surface. The HEPA filtered air from the overhead grill flows in a uniform downward movement to minimize the air turbulence. It is this air that provides and maintains a clear air work environment. A percentage of air drawn through the front and back grills of the work surface, is also HEPA filtered and exhausted from the cabinet. Class III cabinets or glove boxes are gas tight cabinets and all operations within the cabinet are conducted through arm-length rubber gloves. Air entering class III cabinets is HEPA filtered and exhaust air is filtered through two HEPA filters in series and exhausted directly to the outside.

Class I and Class II cabinets are partial containment devices which, if used in conjunction with good laboratory practices, can dramatically reduce the risk of operator exposure to biohazardous material aerosols and droplets. Class III cabinets are generally used for extremely hazardous work (e.g. BL 4 labs at the CDC) or experiments with a high potential for aerosolization of an agent that is transmitted by aerosolization.

There is one type of Class I cabinet, four types of Class II cabinets (IIA, IIB1, IIB2 and IIB3) and one type of Class III biosafety cabinet--each differentiated in accordance with the parameters shown in the following chart from the CDC-NIH handbook, "Primary Containment of Biohazards: Selection, Installation and Use of Biological Safety Cabinets".

BSC Class	Face Velocity	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	YES	YES
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 the 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. 70% is exhausted while 30% is recirculated to the cabinet.	YES	YES (minute amounts)
II B2	100	No recirculation; total exhaust to the outside through hardduct and a HEPA filter.	YES	YES (small amounts)
II B3	100	Same as IIA, but plenums are under negative pressure to the room; exhaust air is thimble-ducted to the outside through a HEPA filter.	YES	YES (minute amounts)
III	N/A	Supply air inlets and hard-duct exhausted to outside through two HEPA filters in series.	YES	YES (small amounts)

Proper Use

Start Up

1. Turn off ultraviolet light (if so equipped) as soon as you enter the room.
2. Turn on all blowers and cabinet illumination lights.
3. Allow five minutes of operation to purge system; check flow alarm system audio and visual alarm function if so equipped.
4. Decontaminate readily accessible interior surfaces with a disinfectant appropriate for the agents or suspected agents present.

Shut Down

1. Decontaminate and remove all items from interior work area.
2. Decontaminate readily accessible interior surfaces with a disinfectant appropriate for the agents or suspected agents present.
3. Turn on ultraviolet light if so equipped.
4. Allow five minutes of operation to purge system.
5. Turn off cabinet blower.

Moving/Installation

Biological safety cabinets must be decontaminated prior to moving. In order to ensure filter integrity, the equipment must be recertified after the cabinet is installed at its new location. The PI must arrange for this work well in advance, in order for Facilities Management personnel to meet your schedule.

Decontamination and Maintenance

The PI is responsible for cleaning and decontaminating his or her biological safety cabinets. Facilities Management personnel will disconnect the cabinet and label when the cabinet was disconnected and decontaminated. If the safety cabinet is equipped with a UV light, the bulb should be checked and cleaned every other week for dust. Replace the bulbs every three months to ensure the proper function.

Certification

All biological safety cabinets that are used for handling biohazards must be recertified annually. Contact Jack Mutchler in Facilities Management 626-6880 if you have a safety cabinet that needs recertification.

Centrifuges

Hazards associated with centrifuging include mechanical failure (e.g., rotor failure, tube or bucket failure) and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users should be properly trained, and operating instructions that include safety precautions should be prominently posted on the unit.

Aerosols are created by practices such as filling centrifuge tubes, removing plugs or caps from tubes after centrifugation, removing supernatant, resuspending sedimented pellets and by the very process of centrifugation. The greatest aerosol hazard is created if a tube breaks during centrifugation. To minimize the generation of aerosols when centrifuging the following procedures should be followed:

Always balance buckets, tubes and rotors properly before centrifugation.

Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.

Add disinfectant to the space between the tube and the bucket to disinfect material in the event of breakage during centrifugation.

To minimize the generation of aerosols when centrifuging biohazardous material, the following procedures should be followed:

If the centrifuged specimen contains biohazardous material, open the centrifuge tubes inside a BSC with the tube pointed away from you.

If centrifuging biohazardous materials that can be transmitted via aerosol, fill and open centrifuge tubes, rotors and accessories in a biosafety cabinet (BSC). Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.

Do not decant or pour off supernatant of tubes containing biohazardous materials. Use a vacuum system with appropriate in-line reservoirs and filters.

Work in a BSC when resuspending sedimented material from a biohazardous source. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.

Small low-speed centrifuges may be placed in a BSC during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines. Manufacturers' recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.

Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, an appropriate chemical disinfectant must be used to decontaminate them.

Aerosol-Creating Equipment

The use of blenders, ultrasonic disrupters, grinders and lyophilizers can result in considerable aerosol production. This equipment should be used in a BSC when working with biohazardous materials.

Blenders

Safety blenders are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation and to withstand sterilization by autoclaving. Test blender rotors with sterile saline or dye solution to determine if they are leakproof prior to use with biohazardous material. The use of glass blender jars is not recommended because of the breakage potential. If they must be used, glass jars should be covered with a polypropylene jar to prevent spraying of glass and contents in the event the blender jar breaks. A towel moistened with disinfectant should be placed over the top of the blender during use. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle and then open in a BSC. The device should be decontaminated promptly after use.

Sonicators and French Presses

Sonication of living microorganisms is potentially a major source of aerosols. Whether using a sonicating bath or probe sonicator, precautions should be taken to protect personnel. Ordinarily, this will be done by performing the sonication in a biosafety cabinet or glove box. It is prudent to consider all surfaces in the vicinity of the sonicator to be contaminated following its use, and they should be thoroughly disinfected.

The use of French pressure cells requires similar caution. The greatest potential for aerosols is at or near the end of a pressing cycle, when air bubbles at the top of the column of suspension can escape with little or no warning. This can result in microaerosols, which will contaminate the work area, but also in macroaerosols which can effectively inoculate the mucus membranes and conjunctivae of the operator. Due to the size of the press, it is usually impractical to perform this operation in the hood. Thus, one should avoid pressing live organisms which are human pathogens. Operators should use face shields or other eye protection.

Arcing, which sometimes occurs during electroporation of bacteria, can also cause aerosols. These range from minimal spattering of the bacteria:DNA solution to major broadcast of potentially infectious material when a cuvette shatters. The shields supplied with most electroporators are usually sufficient to protect the operator from flying plastic and gross contamination, but will not contain microaerosols. Thus, if one must electroporate bacteria which are likely to cause human disease if ingested or inhaled, it

should be done in a biosafety cabinet.

Cell Concentrators

Cell concentrators are also employed in laboratories as a means of handling viable organisms. There are two principal type of cell concentrators. The first involves the removal (through evaporation) of liquid from solid material thereby increasing the concentration versus volume. The second involves the retention of the solid material on the surface of a filter and the subsequent harvesting of the material from the filter surface. The following safety rules should be applied when using such apparatus:

1. Before starting, check all of the equipment to be used for signs of stress or fatigue. Pay close attention to tubing and glassware
2. When possible conduct the procedure under a hood.
3. Upon the completion of the run, thoroughly sanitize the apparatus before the next experiment.
4. For rotary type concentrators, make sure the load is balanced.
5. If a vacuum is to be used, make sure the appropriate exhaust filter is present on the vacuum line to prevent contamination (normally a 0.22 μ m hydrophobic filter).
6. Do not exceed recommended pressures or speed for operation of equipment.

Lyophilizers and Ampoules

Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material should be loaded in a BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized and vapor traps should be used wherever possible.

Opening ampoules containing liquid or lyophilized culture material should be performed in a BSC to control the aerosol produced. Gloves must be worn. To open, nick the neck of the ampoule with a file. Wrap it in a disinfectant soaked towel. Hold the ampoule upright and snap it open at the nick. Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the contents without bubbling and withdraw it into a fresh container. Discard the towel and ampoule top and bottom as biohazardous material waste.

Ampoules used to store biohazardous material in liquid nitrogen have exploded causing eye injuries. The use of polypropylene tubes eliminates this hazard. These tubes are available dust-free and presterilized, and are fitted with polyethylene caps with silicone washers. Heat sealable polypropylene

tubes are also available.

Protecting Vacuum Systems when Filtering Biological Materials

The aspiration of tissue culture media from cultures and supernatants from centrifuged samples into primary collection flasks is a common laboratory procedure. Protection against pulling biological aerosols or overflow fluid into the vacuum system is necessary. An overflow flask and a cartridge type filter provide protection for the vacuum line.

The cartridge type filter must be hydrophobic and have a pore size of 0.2 μm for an effective barrier to virus and bacterial aerosols.

For assembling the apparatus, flexible tubing is used of appropriate inside diameter for the flask and filter fittings and of sufficient wall thickness for the applied vacuum. Filter flask of capacities from 250 to 4,000 ml may be used for the overflow flask depending on the amount of fluid that could be aspirated out of the collection flask.

The overflow flask contains a disinfectant solution appropriate for the biological material under study. Bubbling of air through the disinfectant can cause foam which can shut off the vacuum if it reaches the filter.

If the filter becomes contaminated and requires exchanging, the filter and flask can be safely removed by clamping the line between the filter and the vacuum source. The filter and flask should be autoclaved before the filter is discarded. A new filter may then be installed and the assembly replaced.

Pipetting

Pipetting is the act of transferring, measuring or dispensing a liquid through a small piece of apparatus typically consisting of a narrow tube. Pipets can be constructed of a variety of glass or plastic materials. Liquids can be drawn into the pipet through use of hand-held bulbs, manual pipet aids, motorized pipet aids, or various other vacuum sources.

Pipetting is a routine function in most laboratories and therefore the safety concerns must not be overlooked. The following safety rules should be followed when using pipets:

1. Never pipet by mouth.
2. Visually inspect the pipet prior to inserting it into any pipet aid. Make sure the pipet does not have any cracks
3. Always dispose of pipets in hard walled containers. If not available, use double hazardous waste bags. Avoid over filling.
4. Routinely clean and inspect pipet aids and bulbs. Damaged pipet aids and weakened bulbs should be discarded.

5. Motorized pipet aids should have some type of filter (typically 0.45 μm or 0.22 μm) to prevent liquid from accidentally being drawn into the housing.

Syringes and Needles

The use of needles and syringes should be limited to situations in which there is no alternative. Syringes and needles account for more laboratory and hospital injuries than any other source, and must be used with extreme care. There is no glove or other safety device currently on the market which will protect a person from a needle stick. Therefore, safety is totally user-dependent. The following rules should be strictly adhered to:

1. Never recap a needle. Do not bend, break or shear needles.
2. Dispose of needles in the appropriate hard-walled container.
3. When uncapping needles, remove sheath away from the body.
4. Avoid removing air bubble by tapping the syringe and expelling into the air. Expelling air in this manner can generate potentially dangerous aerosols. If removal of air is essential, expel air into a premoistened paper towel.
5. Always use the smallest needle which will adequately accomplish the task at hand. The greater the needle, the bigger the potential wound, and the greater the potential inoculation.
6. When injecting and removing samples from a vial, pay careful attention. Avoid forcing the needle through the septum. If the needle can be inserted into the vial placed in a rack or holder, do so.
7. When injecting and removing samples from animals, make sure the animal is properly immobilized prior to the procedure.
8. Avoid the use of glass syringes when possible. Use pre-sterilized, disposable plastic syringes. Do not reuse needles.
9. Avoid using syringes as pipets. If a sample must be removed from one container to another, use a pipet or a micropipette
10. To avoid recapping a syringe containing a vital sample, either place the needle and syringe into a suitable glass tube and secure or expel contents into a suitable container and store. Do not store samples in syringes with needles unless the needle is well protected.
11. Report all needle stick injuries to Risk Management and Safety.