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E. Biosafety Ventilation Equipment

Biological safety cabinets are the principal equipment used to provide physical containment. They are used as primary barriers to prevent the escape of aerosols into the laboratory environment. This is an important function because most laboratory techniques are known to produce inadvertent aerosols that can be readily inhaled by the laboratory worker. Certain cabinets can also protect the experiment from airborne contamination. The selection of a Biological Safety Cabinet is based on the potential of the laboratory technique to produce aerosols and the need to protect the experiment from airborne contamination.

Three types of Biological Safety Cabinets are used in the microbiological laboratory: the Class I, the Class II, and the Class III cabinets. They are as follows:

1. The Class I Biological Safety Cabinet

- a. The Class I cabinet is a ventilated cabinet that may be used in three operational modes: (1) with a full-width open front, (2) with an installed front closure panel without gloves, and (3) with an installed front closure panel equipped with arm-length rubber gloves. Materials may be introduced and removed through the panel opening and, if provided, through the hinged front view panel or a side UV air look. Lights, vacuum, gas (do not provide if cabinet is to be operated, sealed, and with gloves installed), water, and drain can be provided. The materials of construction shall be selected to withstand wear, corrosive action of gases and liquids, and decontaminants. Room air flowing into the cabinet prevents the escape of airborne contaminants from the cabinet work area. It flows across the work space, over and under a back wall baffle, out through a HEPA filter and blower in an overhead duct to the building air exhaust system or outdoors. When operated with a full-width open front, a minimum inward face velocity normal to the work opening of at least 75 fpm is required.
- b. Protection is provided to the user and the environment, but not to the product (experiment). A wide range of activities is accommodated using equipment as varied as pipetting aids, burettes, pH meters, sonicators, shielded centrifuges, blenders, and lyophilizers. Chemical carcinogens and low levels of radioactive materials and volatile solvents can be used in Class I cabinets with minimum face velocities of 100 fpm. When these materials are used in the Class I cabinet, a careful evaluation shall be made to determine that concentrations do not reach dangerous levels or cause problems of decontamination of the cabinet.
- c. The cabinet is a partial containment unit. Its primary barrier-function can be compromised by the pumping action of sudden withdrawal of the hands, the opening and closing of the room door, or rapid movements past the front of the cabinet. Aerosols created in large quantities may overcome even higher face velocities. Also, the cabinet does not protect the experimenter's hands and arms from contact with

hazardous materials. Such protection is dependent on technique and the use of gloves and other protective clothing.

2. The Class II Biological Safety Cabinet

- a. The Class II cabinet is commonly known as a laminar airflow Biological Safety Cabinet. Class II cabinets have a front opening for access to the work space and for introduction and removal of materials. Airborne contaminants in the cabinet are prevented from escaping across this opening by a curtain of air formed by (1) unfiltered air flowing from the room into the cabinet and (2) HEPA filtered air supplied from an overhead grille in the cabinet. This curtain of air also prevents airborne contaminants in the room from entering the work space of the cabinet across the front opening. The curtain of air is drawn through a grille at the forward edge of the work surface into a plenum below. Air from this plenum is HEPA filtered and recirculated through the overhead grille down into the cabinet. A portion of this filtered air is used to maintain the air curtain and the remainder passes down onto the work surface and is drawn out through grilles at the back edge of the work surface. The HEPA filtered air from the overhead grille flows in a uniform downward movement to minimize air turbulence. It is this air that provides and maintains a clean-air work environment. A percentage of air drawn through the front and back grilles of the work surface, which is equal to the flow of room air into the cabinet, is also filtered by HEPA filters and exhausted from the cabinet.
- b. The selection of utility services and materials of construction are similar to those for Class I cabinets.
- c. There are two types of Class II cabinets, A and B. These differ principally as to:
 - i. Vertical dimension of the front opening
 - ii. Proportion of air recirculated
 - iii. Velocity of airflow to work surface
 - iv. Manner of discharge of exhaust air
 - v. Whether contaminated air plenums are under positive pressure
- d. The Type A cabinet has a fixed front access opening. The inward face velocity through the front opening is at least 75 fpm. Contaminated air plenum are normally operated at positive pressure. The cabinet operates with a high percentage (approximately 70%) of recirculated air. The Type A cabinets can be operated with recirculation of the filtered exhaust air to the room in which they are located. This minimizes extra demand on supply and exhaust air systems unless the buildup of heat and odor from the recirculated exhaust air requires otherwise.
- e. Type B cabinets do not recirculate their exhaust air to the room. They have a vertical sliding sash rather than the fixed opening of the type A. Inward air velocity of 100 fpm is attained at an 8" sash opening. The cabinet operates with a low percentage (approximately 30%) of recirculated air.
- f. Type A and B cabinets are partial containment units with the same limitations as Class I cabinets. These cabinets provide protection to the user, environment, and product (experiment). Activities are accommodated that use pipetting aids, burettes, pH

meters, sonicators, blenders, lyophilizers, and shielded centrifuges. The Type B cabinets can be used with dilute preparations of chemical carcinogens, of low-level radioactive materials, and of volatile solvents when the face velocity of 100 fpm is maintained. When these materials are used, however, a careful evaluation shall be made to determine that concentrations do not reach dangerous levels or cause problems of decontamination of the cabinets. The Type A cabinets cannot be used with toxic, explosive, flammable, or radioactive substances because of the high percentage of recirculated air.

3. The Class III Biological Safety Cabinet

- a. The Class III cabinet is a totally enclosed ventilated cabinet of gas-tight construction. Operations within the Class III cabinet are conducted through attached rubber gloves. When in use, the Class III cabinet is maintained under negative air pressure of at least 0.5" water gauge. Supply air is drawn into the cabinet through HEPA filters. The cabinet exhaust air is filtered by two HEPA filters installed in series or one HEPA filter and an incinerator. The exhaust fan for the Class III cabinet is generally separate from the exhaust fans of the facility ventilation system.
- b. Materials are introduced and removed through attached double-door sterilizers and dunk baths with liquid disinfectants. The usual utility services can be provided, but not gas. Liquid wastes go to a holding tank for appropriate decontamination before release into "common" sewage lines. Stainless steel is the usual construction material. Modular designs provide for inclusion of refrigerator, incubator, deep freeze, centrifuge, animal holding, and other special cabinet units.
- c. The Class III cabinet provides the highest level of personnel and environmental protection. Protection is also provided to the product (experiment). Most laboratory activities can be accommodated: the usual cultivation of microorganisms, fertile eggs, tissue cells; microscopy; serology; animal dissections and injections; experimental aerosol exposures; various physical measurements; and many others on a small-to-large scale. Selected gaseous atmospheres can be maintained at desired humidity and temperature conditions.
- d. The Class III cabinet protection can be compromised by puncture of the gloves or accidents creating positive pressure in the cabinet. Flammable solvents shall not be used in the cabinets unless a careful evaluation has been made to determine that concentrations do not reach dangerous levels. When required and determined safe, these materials shall only be introduced into the system in closed, non-breakable containers. These materials shall not be stored in the cabinet. Electric heaters are preferred over portable, canned-gas heaters. Flammable gas shall not be piped to the units.

4. Laminar Flow Clean Air Cabinet

This cabinet is not suitable for work with biohazards. Personnel are exposed to contaminated air because the cabinet's positive pressure allows air to flow out of the cabinet. Such units are suitable only for use with known "clean" materials where product protection is the only objective.

5. Certification of Biological Safety Cabinets

The capability of biological safety cabinets to protect personnel and the environment from exposures to potentially hazardous aerosols is dependent on both the ability of the laboratory worker to use the cabinet properly and the adequate functioning of the cabinet itself. A biological safety cabinet shall never be used to contain hazardous materials unless it has been demonstrated to meet certain minimum safety specifications.

Certification of the cabinets for minimum safety specifications is required whenever (1) a new cabinet has been purchased and installed, but before it is used, (2) after it has been moved or relocated, and (3) at least annually. This service is provided by outside companies. Please consult Occupational and Environmental Safety for names of companies.