

PRIMARY CONTAINMENT (CHAPTER 3)

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ABSTRACT

Protection of laboratorians and their immediate environment from hazardous research materials (primary containment) requires meticulous preparation and execution of appropriate protocols and practice. Just as important is paying attention to detail in the selection, installation, proper use, maintenance and certification of proper operation of safety equipment. In order to accomplish this a basic understanding is necessary of primary containment equipment and its performance. This chapter deals with primary containment, Biological Safety Cabinets, suit rooms, glove boxes and the certification and proper use of these types of primary containment equipment.

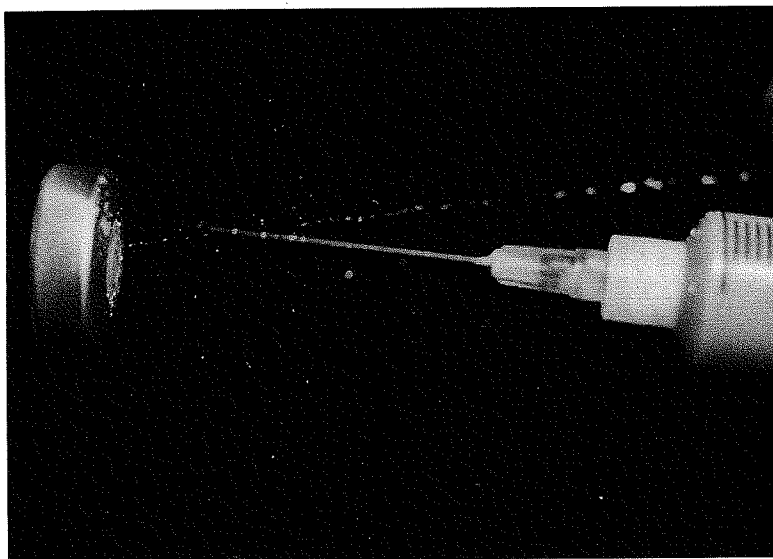
INTRODUCTION

Need for primary containment

The nature of laboratory acquired infections and the mechanisms associated with their occurrence (Richmond and McKinney, 1993) make clear the need for laboratorians to be protected from the microbiological agents with which they work. Virtually every activity in the laboratory gives rise to an aerosol of some magnitude (Chatigny, 1969). The improper use of a syringe, needle and septum (Figure 1) is an example of this. Any droplets in the photograph that are large enough to be seen are too large to qualify as aerosols. However, for every droplet that can be seen there are many of aerosol size which are colloidal in nature and capable of spreading microbial agents far and wide. Hence the need for primary containment.

FIGURE 1

Aerosol creation by improper use of needle and syringe.



This chapter is from *Anthology of Biosafety I: Perspectives on Laboratory Design*. This publication is available for purchase from the ABSA National Office, 1202 Allanson Road, Mundelein, Illinois 60060-3808.

Role of the HEPA filter in biocontainment

Aerosols are removed from the laboratory work space using controlled air flow of sufficient velocity and volumetric flow rate to capture aerosols and sweep them into exhaust ducts and away from the worker. In order to remove microbial agents from the moving air within which they are entrained, a filtration device is required that will satisfy two seemingly mutually exclusive requirements. The filter must provide enough air flow at low pressures (about 2" w.c.) to achieve capture velocity for aerosols in the work area. It must also efficiently filter out submicron particles.

The HEPA filter is capable of meeting the air-flow requirement while filtering out the most penetrating particle size (nominally 0.3μ) with a minimum efficiency of 99.97%. This means that the HEPA filter retains particles that are larger than 0.3μ with more efficiency than 99.97%. It also means that, below the most penetrating particle size, the smaller the particle is, the more efficient the HEPA filter is in retaining it (First, 1998). Thus, the controlled airflow of the cabinet together with the HEPA filter make containment of microbial agents possible.

Role of Ventilation in Chemical Vapor Containment

Efficient as HEPA filters are at filtering out particulates, gases and vapors go right through them. If potentially hazardous chemicals that may vaporize are to be used in small quantities as an adjunct to the microbiological work, adequate ventilation is required to dilute and carry vapors away (Barkley, 1972). The exhausted air should pass through air cleaning devices appropriate for the compounds being used.

PRIMARY CONTAINMENT

The Goal of Primary Containment

The goal of Primary Containment is to protect personnel and their immediate environment from exposure to infectious agents (Richmond, 1993).

The How of Primary Containment

Primary Containment is accomplished by preventing the migration of contaminants away from the site of their generation. In addition to controlling aerosols, spread of microscopic infectious agents by

contact must also be controlled. The challenge here is that the amount of material that can cause a problem is often so small that it cannot be picked up by the human senses. Therefore, competent procedures using capable and correctly operating equipment to prevent spread of biohazardous material are essential.

Risk Assessment

Success of primary containment efforts rests upon an adequate risk assessment and transformation of the knowledge obtained into use of the practices and equipment required to work safely at the level of risk that is identified (Richmond, 1995). Risk levels of various hazardous microbiological agents and the laboratory containment levels appropriate for working with them are classified as Biological Safety Levels (BSL) 1 through 4 (Richmond, 1993).

Practices

Effective Primary Containment requires rigorous application of appropriate practice (following the practices and procedures in the laboratory operations manual) and proper use of engineering controls. Guidelines for use of personal protective equipment and engineering controls are available (Richmond, 1995; Fleming, 1995). Ineffective practices can quickly nullify any advantages made possible by the kinds of equipment that we are about to discuss.

Interrelationship between Containment Equipment and Facilities

There must be space for Biological Safety Cabinets to be located out of traffic areas and out of the influence of cross drafts from ventilation diffusers, swinging doors and the like. It has been well documented that the performance of BSCs can be degraded by what is going on around them (Rake, 1978; Clark, 1990).

The laboratory must be viewed as a secondary containment barrier and designed accordingly. Building exhaust systems provided for BSCs must have special attention given to the cabinets' constant flow and static pressure requirements. Utilities (especially gas) need shut-off valves outside the BSCs and protection on lines that could allow biohazards to escape, such as drains and vacuum lines.

Personal protective equipment (PPE) such as gowns, gloves and pipetting aids must be available and properly used. (Kuehne, 1995). For example, if gloves are contaminated while working in a Biological Safety Cabinet and are not properly removed before leaving the cabinet, primary containment may be breached no matter how well the Biological Safety Cabinet performs.

Adequate facilities for limiting access to the laboratory, for sterilization and decontamination of equipment and materials and adequate ventilation of the laboratory are also important to successful primary containment (Richmond, 1993).

BIOLOGICAL SAFETY CABINETS

Rationale for and History of Biological Safety Cabinets

The rationale for the Biological Safety Cabinet (BSC) is to provide a readily cleanable workstation within which contamination generated by working with infectious agents can be contained. Any aerosols generated by the work are immediately swept out of the work area and onto HEPA filters by the airflow of the cabinet. Additionally, contaminated materials and equipment can be packaged within the cabinet. The exterior of the package can then be decontaminated before removal from the cabinet. This will prevent spread of contamination by contact. BSCs have been modified to facilitate

this process (Stimfel, 1991).

The Biological Safety Cabinet is a fairly recent development. While precursors of BSCs were worked on as early as 1909, the Class III cabinet as we know it was developed in the late 1940s and the Class I BSC was first reported on in the mid 1950s (Kruse, 1991). The Class II cabinet was first microbiologically performance tested in the late 1960s (Coriell, 1968; McDade, 1968).

Class I BSC

The sweeping of airborne particulates out of the BSC work area is clearly illustrated by the smoke patterns in Figure 2. The Class I BSC utilizes a remote exhaust fan to draw room air into the cabinet through a work access opening, through the work area and then out through a HEPA filter at the top of the cabinet. It is to be operated with a minimum of 75 fpm intake air velocity through the work access opening (Richmond, 1993). Environmental protection is provided by the exhaust HEPA filter. Product in the Class I cabinet is not protected from contamination in the intake air. The containment protection factor (leakage factor) for a Class I has been reported to be 10^{-4} to 10^{-7} depending on the design of the cabinet, the amount of activity in the cabinet, the operations performed and how the work is done (Kuehne, 1995). The higher protection factor was achieved with a glove port panel on the work opening.

FIGURE 2
Smoke being swept away in a Class I Biological Safety Cabinet.

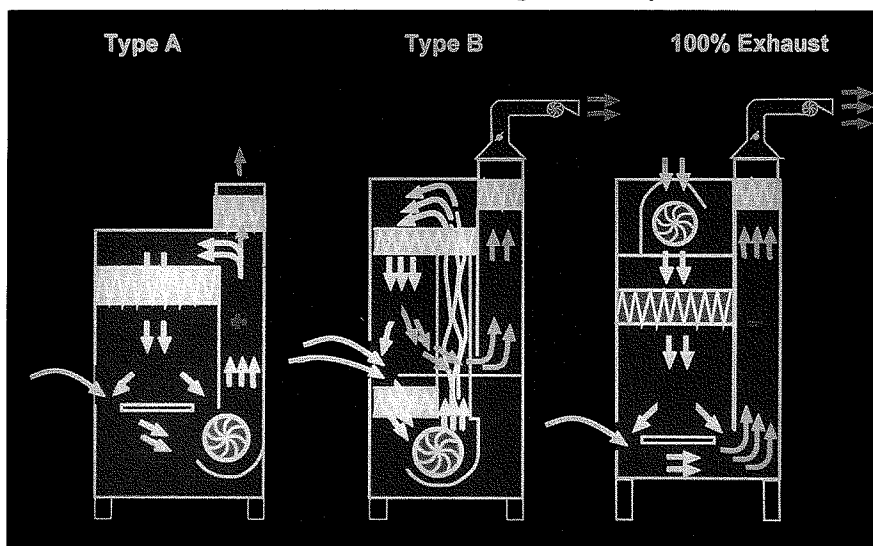


Class II BSC

The Class II BSC was developed to provide both primary containment (personnel and environmental protection) and product protection by means of HEPA filtered air flowing down through the work area, intake air that does not enter the clean work area and HEPA filtration of air leaving the cabinet (Figure 3). The fundamental criterion for a cabinet to qualify as a Class II BSC is successfully passing the microbiological aerosol tracer performance tests for personnel, product and cross contamination protection. These tests are now defined in NSF Standard

49 (NSF 1992). Protection factors of 10^{-4} to 10^{-7} are also reported for Class II BSCs. The same caveats of cabinet design, amount of activity, the operations performed and how the work is done apply to the Class II BSC also (Kuehne, 1995). Additionally, each design of Class II BSC has a unique performance envelope of intake and supply air velocities within which it passes the microbiological tests. (Jones, 1990). Passing the NSF personnel protection test results in a protection factor of 10^{-5} , as calculated and also required in the UK standard (British Standards Institution, 1979).

FIGURE 3
Comparison of the original Type A, NCI-design Type B and 100% exhaust Class II Biological Safety Cabinets.



The first classification of a Class II Biological Safety Cabinet was the Type A (formerly called Type 1) detailed in a purchase specification from the National Institutes of Health (NIH, 1974). This cabinet has a minimum intake air velocity of 75 fpm, microbiologically contaminated plenums that are under positive pressure to the room, recirculates approximately 70% of the cabinet airflow and returns HEPA filtered exhaust air back to the room from a common plenum (Figure 3-Type A). This cabinet was designed for microbiological work and performs well handling microorganisms, which are particulate in nature. However, if vapors or gasses are used in the Type A cabinet, they will pass through HEPA filters and build up in the work area

and will also be exhausted into the room.

The rationale for the original Class II Type B (formerly called Type 2) Biological Safety Cabinet was to develop a BSC that could be used for containing volatile materials and not be limited to containment of basic microbiological techniques (Barkley, 1972). The resulting cabinet (Figure 3-Type B) was described in a purchase specification from National Cancer Institute (NCI, 1976). Some of the design features used to accomplish this goal were: exhausting a higher percentage of cabinet air and recirculating less than a Type A cabinet, bringing the total airflow of the cabinet down through the work area for better ventilation, requiring a building exhaust blower to pull the exhaust air out of the

cabinet, using a dedicated plenum for air exhausted at the back of the work surface, placing a HEPA filter immediately below the work surface, keeping ducts carrying unfiltered air under negative pressure, ducting air exhausted from the cabinet outside of the building and having an average intake air velocity of 100 feet per minute. While this Type B cabinet design was a considerable improvement over the Type A, it still recirculated roughly 30% of the cabinet airflow back into the work area.

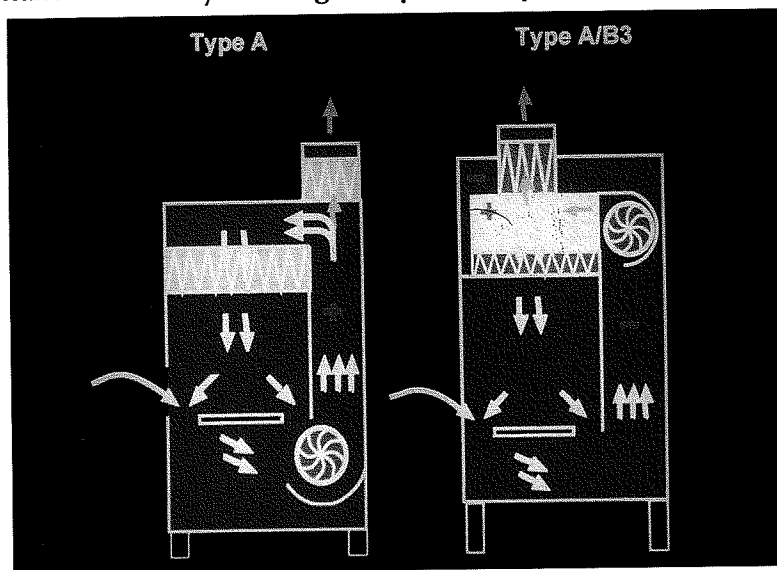
The Class II 100% exhaust Biological Safety Cabinet (Figure 3-100% Exhaust) was developed to complete the spectrum of vapor handling capability: approximately 70% cabinet air recirculation in the Type A, approximately 30% recirculation in the

Type B and 0% recirculation in the 100% exhaust cabinet. How these types of Class II BSCs handle vapors differently has been quantitatively described (Stuart, 1983).

In the meantime, a Type A negative pressure cabinet evolved that has 100 fpm intake air velocity and plenums adjacent to the room under negative pressure. The original Class II Type A and this modified Type A design, now called A/B3, are compared in Figure 4.

NSF Standard 49 (NSF, 1992) contains definitions of four types of Class II Biological Safety Cabinets. These definitions can be best understood by relating them to the earlier types:

FIGURE 4
Comparison of the original NIH-design Type A and the later Type A having 100 fpm intake air velocity and negative pressure plenums adjacent to the room.



NSF Type A includes both the original NIH-design and the negative pressure plenum cabinet (Figure 4).

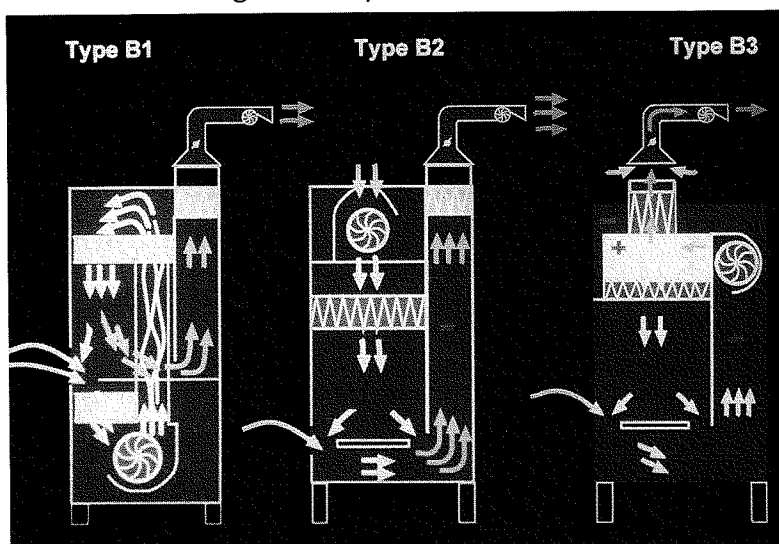
NSF Type B1 includes the NCI-design Type B. However, it does not require certain of the NCI-design features such as the HEPA filter immediately below the work surface and some of the specific airflow criteria that were included in the NCI specification.

NSF Type B2 is the 100% exhaust cabinet.

NSF Type B3 is the Type A cabinet which maintains a minimum of 100 fpm intake air velocity and has all biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure. When this Type A cabinet discharges all air leaving the exhaust HEPA filter to the outdoor atmosphere it becomes a Type B3. The original NIH-design Type A **cannot** become a Type B3 when its exhaust air is discharged to the outdoors.

FIGURE 5

Comparison of the three Class II Biological Safety Cabinets that are called Type B in NSF Standard 49.



The three Class II BSCs that are called Type B cabinets are compared in Figure 5.

Here we have four types of cabinets that pass the microbiological tests to qualify as Class II Biological Safety Cabinets for use at BSLs 1-3. Three of them may be used with varying minute amounts of volatile materials according to the NSF use statements (NSF, 1992). The Type A cannot. In order of increasing vapor handling capability they are: Type B3, B1 and B2. Depending on what is being used in the Type B cabinets, exhaust air may require more treatment than mere HEPA filtration (NCI, 1975).

Class III BSC

The Class III BSC is a specialized glove box type enclosure that provides a physical barrier between personnel and the materials they are working with. It is covered in the chapter of this book entitled Role of the Class III Biological Safety Cabinet in Achieving BSL-4 Containment.

Modifications of BSCs

When standard BSCs will not accommodate certain applications they can be modified to house equipment such as centrifuges (Figure 6), carboys, microscopes, etc. Specialized BSCs can be designed to serve as animal cage dump stations, robotic work areas and the like. Microbiological testing must be done on modified units to ensure that performance has not been compromised.

SUIT ROOMS

BSCs

An alternative to using Class III Biological Safety Cabinets in a BSL-4 laboratory is to use Class I or II cabinets and provide positive pressure air-supplied suits for the personnel.

Suit

Since the primary containment provided by the Class I or II cabinets is not "absolute," the "space suits" are used as a precaution against a possible breach of primary containment in the cabinets. The suits (Figure 7) provide absolute protection to the personnel while allowing more freedom of movement within a room than working in a Class III cabinet.

Room

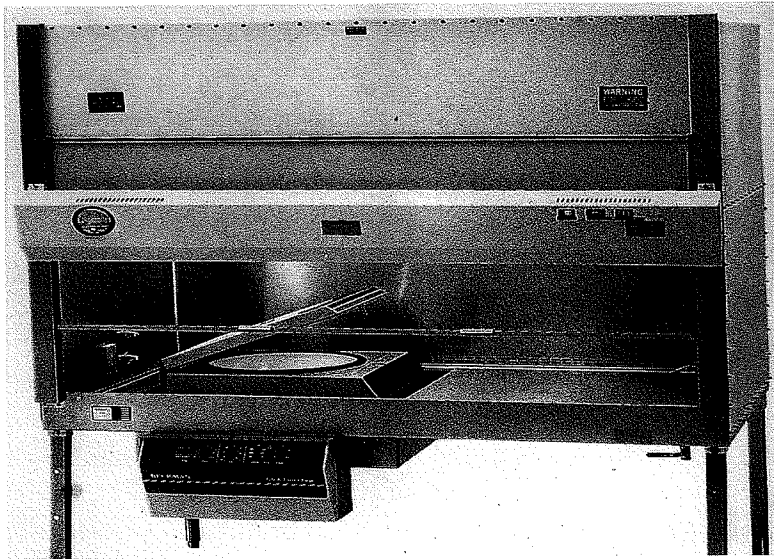
The BSL-4 suit room takes on the characteristics of a large Class III cabinet (Kuehne, 1995). Since the open-fronted BSCs are partial containment devices, if BSL-4 agents are in use, the room must ensure the primary containment.

GLOVE BOXES

Chemical Glove Boxes

Glove boxes are used for primary containment of many varied hazardous materials and come in

FIGURE 6
Example of Cabinet modification for a specific application.



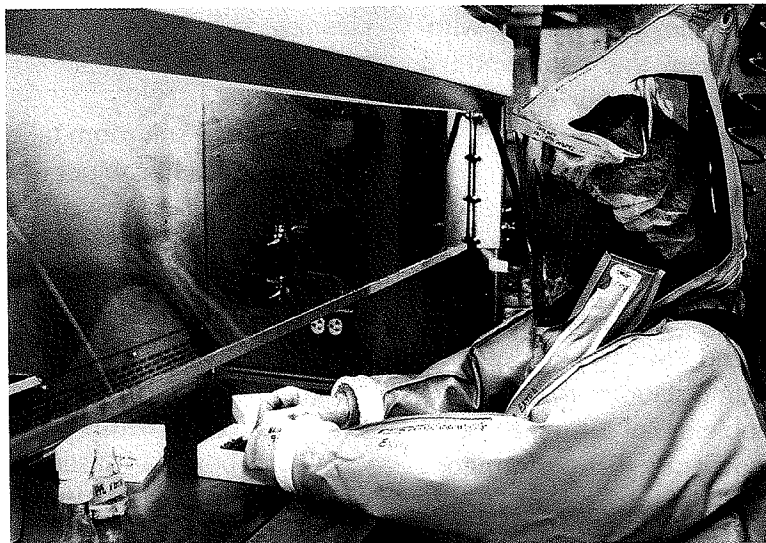
different shapes and sizes depending on the nature of the material to be contained and the activities that are to take place in the box. Glove boxes to house robotic handling of radioactive materials are designed differently than boxes used for weighing raw chemical carcinogens or highly potent pharmacological materials.

Bioclean Systems (Isolators)

Bioclean systems are specialized glove box systems designed to provide product protection, which

could be looked at as primary containment in reverse, and are called isolators (Figure 8). Here the goal is to provide a mini-environment within which sterile materials can be manipulated aseptically. A typical application is enclosure of a pharmaceutical filling line to enable the aseptic transfer of sterile material from bulk to vials, including stoppering and capping. All motors and machinery are located outside the isolator with nothing inside that does not absolutely have to be there. Double ULPA (ultra-low-penetration-air) filtered air is provided to

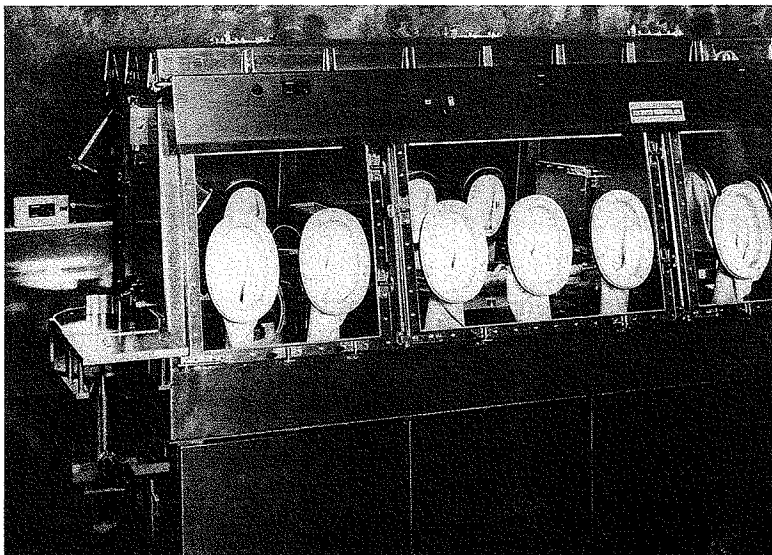
FIGURE 7
Working in a positive pressure air-supplied suit.



the box to keep it flushed with clean air and under positive pressure. The vial exit port (mouse hole) is designed to prevent contaminated air from entering the isolator and usually exits into a restricted access barrier which also contains clean air. The interior of the isolator is sterilized with an agent such as vapor phase hydrogen peroxide. Materials and equipment

are passed in through double-door high intensity UV units or through docking devices that do not break the integrity of the aseptic enclosure. Particulate counting and microbiological sampling are used to monitor the performance of this equipment (Wagner, 1995).

FIGURE 8
An example of an isolator.



Bioclean Systems Providing Containment

Isolator technology has also been used to provide primary containment of a hazardous potent compound while at the same time maintaining an aseptic environment for a filling line (Figure 9). This negative pressure isolator has been sterilized and then operated for 6 months with no detectable contamination found (Senour, 1997).

CERTIFICATION

Enough testing and verification of proper operation must be done to provide confidence in the continuing proper operation and the expected performance of these systems.

Decontamination

Decontamination of the equipment before certification is an important part of primary containment. "Decontamination is mandatory when maintenance work, filter changes, and performance tests

require access to any contaminated portion of the cabinet." (NSF, 1992) All interior surfaces of the cabinet should be surface decontaminated with an appropriate disinfectant before any testing is done. Gaseous decontamination must be done before removing panels to enter any area potentially contaminated with biohazards. Gaseous decontamination is usually done by heating paraformaldehyde flakes in a sealed cabinet to release formaldehyde gas. This must be done carefully to prevent formaldehyde leakage into the laboratory and to achieve the intended kill of infectious agents (Fink, 1988; Abraham, 1997).

Biological Safety Cabinets

Just as improper use and practice can compromise the primary containment of Biological Safety Cabinets, improper equipment operation can undo the effort as well. Class II BSCs have been shown to provide little or no protection if their air flow balance wanders out of their performance envelope

FIGURE 9
A negative pressure containment isolator.



(Jones, 1990). To enjoy primary containment performance of BSCs, cabinet integrity, correct air flows, airflow smoke patterns and HEPA filter integrity must be maintained. Certification is the testing in the field following the guidelines in Annex F of NSF Standard 49 (NSF, 1992) to demonstrate that the cabinet is operating essentially the same as its model-mate was when it was microbiologically tested. It is recommended that certification be done at installation, after repair or relocation and at least annually to ensure proper operation (Richmond, 1995). Tests related to worker comfort and safety: electrical (resistance to ground, chassis current leakage, polarity and GFI trip), noise, lighting and vibration are also included in Annex F of NSF 49. This field testing must be done by qualified, experienced personnel (Richmond, 1995). Refer to the chapter in this book entitled Role of the Class III Biological Safety Cabinet in Achieving BSL-4 containment for certification testing that is specific to Class III BSCs.

Suit Rooms

Certification of suit rooms is similar to certifying Class III Cabinets (NCI, 1979).

Glove Boxes

The same principles hold for Class III systems, chemical glove boxes and bioclean isolators. They all have their own combinations of operational requirements including box integrity, correct air flows

and pressures, leak free filter installations and air cleanliness. The equipment must be tested and certified to be operating properly. This is usually called Installation Qualification/Operation Qualification (IQ/OQ). The frequency of testing will be determined by the performance required of the equipment, the ramifications of lack of performance and the operational stability of the system.

PRACTICE

Biological Safety Cabinets

It has already been stated that practice is just as important as the primary containment equipment. Inadequate practice during the use of a BSC can compromise the primary containment offered by the cabinet very quickly. Every laboratory that uses BSCs should have detailed written protocols for working in the cabinets. Sources for the contents of these protocols are: Richmond, 1995; Fleming, 1995, and operator's manuals for the cabinets. Basics include:

- if the cabinet is not left running turn it on and allow a 3-4 minute purge time
- double glove—removable forearm sleeves are recommended
- surface decontaminate the inside of the BSC before starting any work
- have only those items that are required to do the experiment in the cabinet

- be sure to have everything needed in the cabinet—including pipet discard trays
- move smoothly and deliberately while doing the work
- segregate "clean" and "dirty" items
- enter and exit the cabinet smoothly and deliberately, moving straight in and out
- do not exit the cabinet unless absolutely necessary and then only when vessels are closed and outer gloves are properly removed
- observe all warnings and alarms, enclose all materials and surface decontaminate the enclosures before removal from the cabinet
- properly discard all waste—autoclaving if appropriate
- surface decontaminate the work area of the cabinet after it has been emptied
- properly discard or recycle PPEs

See the Chapter 10 for additional information on use of Class III BSCs.

CONCLUSION

A wide range of primary biocontainment equipment is currently available. Proper selection of the equipment will be predicated on the results of the risk assessment for the work to be performed and the agents to be handled. When combined with appropriate work practices, worker safety, product protection and environmental containment can be routinely achieved.

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