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ROLE OF THE CLASS III BIOLOGICAL SAFETY CABINET IN ACHIEVING BIOLOGICAL SAFETY LEVEL 4 CONTAINMENT (CHAPTER 10)

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ABSTRACT

Maximum personnel protection can be achieved in two fundamentally different ways: enclose the investigator in an air-supplied suit, or enclose the biohazardous agent in a primary containment device. This chapter will focus on the use of Class III Biological Safety Cabinets to meet biosafety level 4 containment.

INTRODUCTION

Class III Biological Safety Cabinets (BSCs) have been used for decades to achieve a contained space within which work with highly pathogenic and lethal microorganisms can be performed. Certified and functioning Class III BSCs protect the investigator and the environment. Recent advances in cabinet design have incorporated many ergonomic features that have improved worker comfort. Like Class II BSCs (see Chapter 3), Class III cabinets provide both personnel and environmental protection. Unlike Class II BSCs, the Class III cabinet is not designed expressly to provide product protection; however, since both inflow and exhaust air pass through high efficiency particulate air (HEPA) filters, the interior of the cabinet is relatively particulate free. Use of good microbiological techniques are necessary to help protect the experimental materials and the product.

Investigators work through thick rubber gloves that are securely attached to arm holes in the walls of the cabinet. The nature of the gloves is such that dexterity is reduced, which may increase risk to the investigator to inadvertent glove puncture. However, gloves are currently being made from more flexible materials, thereby improving dexterity considerably. Similarly, reduced ability to manipulate small objects may occur. Another restricting factor is the limited reach afforded by the gloves, even when using "extenders" to push and pull objects along. Centrifuges, microscopes, incubators, animal cages, etc., must be contained within the cabinet. Doors and other portals-of-entry must be modified so that the equipment can be gasket-sealed to the wall, floor, or window of a BSC.

Recent modifications include working in a half-suit that is sealed to the working surface of the cabinet. The investigator is thus partially inside the primary containment device, a configuration that provides more mobility and better access to the materials in the BSC.

Basic Construction

Class III Biological Safety Cabinets (BSCs) are used for protection of workers and the environment from highly infectious and dangerous microorganisms by providing primary containment of the hazardous agents being manipulated. Research materials are not removed without inactivation or proper biocontainment from the physical barrier of the cabinet, which is operated under negative air pressure. The goal here is absolute containment. Therefore, from early on, the basic construction of Class III BSCs was of type 304 stainless steel with a great deal of emphasis placed on the leak tightness of welds, gaskets, penetrations of pipes and wires—the entire component system. Additionally, the cabinets were required to not leak when subjected to a specified halide gas-under-pressure test. In 1965, the U.S. Army explained this with the statement "Since safety is the ultimate factor, it is important that the Class III cabinets be assembled and sealed with the utmost care, with the consideration of

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continuous use over a period of several years" (U.S. Army, 1965).

Given the rationale of our starting point, the attributes of the Class III BSC's basic construction become logical extensions of it. The Class III BSC has been described as a "totally enclosed ventilated cabinet of gas tight construction and offers the highest degree of personal and environmental protection from infectious aerosols as well as protection of research materials from microbial contamination. Class III cabinets are suitable for work with hazardous agents that require BSL 3 or 4 containment." (Richmond, 1993) This ventilated total enclosure requires the following components:

1. A gas-tight box that is usually made of polished stainless steel with coved corners for easy cleaning and effective decontamination.
2. A view screen of laminated safety glass or equivalent that is gasketed to the stainless steel with a solid silicone gasket. Silicone sealant should not be used as a substitute for gasket.
3. Arm length gloves, often neoprene, are attached gas-tight to stainless steel arm ports. The advent of oval arm ports as well as more flexible gloves makes it easier to work in the cabinet.

The cabinet has at least one supply and two exhaust HEPA filters in series to complete the physical primary barrier between the research materials and the outside environment. Sometimes an exhaust incinerator is used in addition to HEPA filtration. After HEPA filters have been verified to be leak-free and shown by testing to have a 99.97% minimum retention efficiency of most penetrating particle sizes of 0.1 to 0.3 um, they are very effective in preventing submicron particles from escaping from the cabinet. At the same time these filters allow sufficient airflow to flush aerosols of microbial research agents out of the enclosure. This includes viruses as well as bacteria and larger microorganisms (First, 1998). Plug-and-seal HEPA exhaust filters can now be used as an alternative to bag-in-bag-out arrangements.

Some Class III cabinet designs include connection ports for decontamination of HEPA filters, incubators, and modules, using formaldehyde gas generating equipment. This design feature allows a more controlled delivery of decontaminating gas than is achieved using frying pans. In a modular Class III cabinet equipped with gas generator ports in each module, decontamination of portions of the Class III cabinet can be achieved without compromising ongoing work in other modules. A biohazardous spill in one module can be decontaminated, neutralized and cleaned in less than 48 hours without shutting down the cabinet line. This feature permits ongoing experiments to be safely completed in modules not affected by spills in other parts of the cabinet line."

A building exhaust fan, preferably with a dedicated independent duct system, is required to pull air through the cabinet. The airflow and static pressure capabilities must enable continuous operation of the cabinet under at least 0.5" water column negative pressure while providing at least 100 fpm velocity of air through a glove port should a glove accidentally come off. It is good to have a HEPA filter situated at the top of the cabinet to provide unidirectional airflow down through the work area. This can provide class 10 or better air cleanliness in the cabinet.

"It is apparent that a BSL 4 agent should be confined entirely within the cabinet system or in a secure container" (Fleming, 1995). Therefore, there should be no direct opening from inside the cabinet to the outside environment. Access must be through a double door autoclave, a disinfectant dunk tank or an air lock pass-through that can be readily decontaminated. (Richmond, 1995). With adequate interlocks and protocols this permits opening an exterior door only into an area that has been decontaminated. This is an important component of the basic construction of a Class III BSC and should be part of the definition of a Class III cabinet. A Class II cabinet modified with a glove panel on the work access opening (Heidt, 1982) would not be considered to be a Class III cabinet. Nor would the cabinet, as shown in the photograph in the Chatigny paper (Chatigny, 1979) qualify as a Class III under this definition. The doors of autoclaves and pass-throughs should be interlocked to prevent both doors from being open at the same time. The autoclave also provides the means for decontaminating all items that need to be removed from the cabinet, such as liquid and solid waste materials.

Modifications of the Basic Cabinet Construction

To maintain BSL-4 containment, the practice is that nothing comes out of the Class III system except by some means of sterilization or disinfection. This has led to the use of a "line" of interconnected Class III cabinets, each designed with modifications that are specific to the activity involved. Cabinets are thus built to the specifications of each individual laboratory program.

Procedures are carefully planned in order to accomplish as much preparatory work as possible outside the cabinet system. The capacity to house all of the equipment required by the laboratory activities involving exposure to the agent are designed into the cabinets in the line. Adaptation of both the cabinet and the equipment is often required.

Examples of modifications include accommodations for centrifuges, microscopes, incubators, refrigerators, freezers, animal housing, animal exposure, necropsy facilities, and controlled atmospheres. Depending on the activities in the laboratory, this can result in a sizable complex of interconnected Class III cabinets (Figure 1).

Most Class III BSCs are fitted with electrical outlets hermetically sealed at the penetration. Air lines leading in or out of the cabinet (e.g., for pressure monitoring, gas supply, or connections to an automatic gas decontamination machine) need to contain an in-line HEPA filter as close to the glovebox penetration as possible. Much of the equipment will have to be modified for inclusion in the cabinet line.

FIGURE 1

An integrated Class III Biological Safety Cabinet line.

Whenever possible, design of equipment to be included in Class III cabinets should allow as much of the equipment used in the cabinet to be serviced from the outside of the cabinet. Examples of this design approach include use of sealed refrigerator/freezer chambers that have condensers, coils and other parts that require routine service or repair located outside of the sealed cabinet. This approach permits work to be performed on this equipment without breaching the biocontainment area of the cabinet. Additional examples of this design approach include external location of centrifuge control panels, incubator electronics and controls, and video monitors which can be easily repaired or replaced as needed.

Secondary Barriers

The Class III cabinet is the primary barrier; the room in which it is placed (along with the support spaces) constitute the secondary barrier. Care should be taken during design and construction to ensure that the ***Biosafety in Microbiological and Biomedical Laboratories*** requirements for BSL-4 secondary barrier are met (Richmond and McKinney, 1993). Depending on the fundamental risk assessments conducted for the agents used and the manipulations employed, the room can be considered to be an augmented BSL-3 facility. By augmented BSL-3, we mean that all requirements for BSL-3 are met, and that some additional requirements are made.

The facility is constructed with monolithic floors with continuous cove moldings extending at least four inches up each wall. Ceilings are hard surfaces that are not part of a dropped ceiling. Walls, extending from floor to ceiling, are finished with a hardened surface, such as epoxy paint.

All penetrations through the floors, walls and ceilings are sealed to prevent air exchange between the containment laboratory and non-containment space. Penetrations for water and steam, air supply and exhaust ducts, electrical conduit and windows are particularly problematic. Insulation must be trimmed back, to within the wall, before sealant is applied; otherwise, air will migrate through the insulation. Window perimeters must be sealed at the window frame, because window frames are not ordinarily airtight. Caulking is also applied to the interior of conduit containing wires or cables. The purpose for sealing these penetrations is two-fold: to prevent migration of airborne or moisture-borne potentially infectious agents out of the containment space in the event of a positive pressurization of the laboratory, and to prevent leakage of the toxic/hazardous gas that may be used during a space-decontamination procedure.

For the same reason, back-flow preventers and sealable dampers should be installed in the supply and exhaust air ducts. Since the laboratory will be maintained under continuous negative pressure, fans must be sized to provide sufficient draw for all the HEPA filters.

The room supply should be interlocked to shut down upon failure of the room exhaust system to preclude the laboratory room from becoming positive in relation to adjacent areas. If redundant exhaust blowers are employed, the secondary exhaust blower should be set to come on line quickly. Even with redundant exhaust blowers installed, the room supply air blower should be interlocked to shut down upon failure of the entire room exhaust system.

The containment facility should be isolated from public areas of the building. Access is controlled through appropriate security systems, such as card identification access, thumb print, etc. Records of access need to be maintained, perhaps with video recordings. Entrance into the facility needs to be through a double-door entry system having self-closing inward-opening doors. Other components of the secondary barrier include:

1. Both a clean clothing change area and a dirty clothing change area, separated by a shower facility;
2. A through-the-wall autoclave, dunk-tank, or other means of safely removing contaminated materials from the laboratory;
3. A decontamination chamber for gas decontamination of large equipment before removal to non-contaminated areas for repair or replacement; and
4. A means for decontaminating liquid effluent from sinks in the laboratory. This can be accomplished chemically, by heat, or other appropriate means.

Interface with the Building

"The space within which a Class III cabinet system is used must be suitable for containment in the event of failure of a cabinet" (Fleming, 1995).

It is important for the project design team to work on the interface of the Class III BSC system with the building as early as possible in the life of the project. There are many things that must be planned for, including the design of the room itself.

The building and the cabinet(s) must be designed such that the cabinet(s) can be unloaded from the truck without damage to the equipment or danger for the personnel involved. Doors, hallways, staircases, elevators and ceilings must be sized so that the cabinets can be moved into place. There must be adequate space around the cabinets to allow unobstructed movement for laboratory and certification activities.

Flow of work within the cabinets and in the room must be planned so that the cabinets can be arranged to provide optimum work efficiency, particularly since even the simplest procedures can become remarkably tedious within the cabinet environment.

Adequate utilities with required seals and filters must be in place for the cabinets and the equipment housed within them including: matching electrical connections of the required voltage, phase and amperage, water (tap and/or treated), gas of various kinds, compressed air and vacuum lines, and properly treated effluent system if there are drains in the cabinets.

There is most often a single exhaust HEPA filter located in an appropriate housing built directly into the cabinet. The second HEPA exhaust filter that is required for Class III cabinets is usually provided in the building exhaust system. This second filter must be properly sized and installed. In some instances the secondary HEPA filter is also built directly into the cabinetry and supplied by the equipment manufacturer.

Special attention must be given to calculation of the flow rate (cfm) and static pressure (inches of water column) requirements for all the air that the building exhaust system will have to handle. This includes air requirements of the cabinets being 100% exhausted to the outside (each one may be different) and of equipment such as cooling air for ultra centrifuges, air changes in animal holding areas and heat load from incubators and refrigeration equipment. The motor/blower will have to handle the static pressure required to pull all this air through the equipment, filters and ducts plus that required to maintain the negative pressure in the cabinets against the negative pressure of the room to hallways and the rest of the building. Therefore, static pressure capabilities of the fans must be carefully designed and closely watched. Interlocked redundant blowers are often used, and uninterrupted power supply (UPS) systems should be considered.

Installation of Class III systems is highly specialized and each must be carefully planned to meet the requirements of the laboratory. Testing for containment and function must be systematically completed before operation in the "hot" mode.

Certification

In order for the Class III system to provide the expected protection for personnel and environment against hazardous microorganisms, the researchers must have the ability to follow safe practices when using the cabinets and the cabinets must be functioning properly. Biological Safety Cabinets should not be used unless they have been demonstrated to meet certain minimum safety specifications. Certification is the testing that is done to demonstrate this. Certification should be done when the cabinet is new (after installation, before it is used), after relocation, and at least annually (NCI, 1979). Certification of Class III cabinets includes the following testing.

Test electrical systems according to UL specifications. Also verify that all alarms and interlocks are calibrated and functioning properly.

Leak tightness for cabinet integrity including gloves, dampers and all ancillary equipment such as autoclaves and pass-through boxes. All of the existing specifications for this test call for the use of freon R-12 refrigerant. The original test was to release 1 ounce of R-12, per 30 ft^3 of cabinet volume, into the sealed cabinet and then bring the pressure in the cabinet to 3" w.c. with compressed air. All welds, gaskets and penetrations using a halide leak detector at a rate of 0.5 inch per second, allowing a leak rate of no more than 0.025 ounces per year (US Army, 1965). This converts to a leak rate of approximately $4.5X10^{-6}$ cc/s. In 1976 the Federal Register published the requirement as "leak rate <1 by 10^{-6} cc/s at 3 in water gage" pressure (NIH, 1976). This was interpreted as pressurizing the cabinet to 3" w.c. directly with R-12 and scanning with the halide leak detector set to alarm at $1X10^{-6}$ cc/s leak rate. This increased the concentration of R-12 in the cabinet a little and decreased the allowable leak rate by about 0.5 log. This test has been commonly used in the industry. The Laboratory Safety Monograph (NCI, 1979) talks about the 1 oz. of R-12 per 30 ft^3 of cabinet volume, pressurizing to 3" w.c. with air and alarming at $1X10^{-7}$ cc/s. Using data from ASHRAE (ASHRAE, 1985) and conversion factors from GE (General Electric, 1965), a comparable test pressurizing the cabinet directly to 3" w.c. with R-12 calculates to be about $3X10^{-7}$ cc/s. Sulfurhexafluoride gas (SF6) can be used as a replacement for R-12 in this testing (Stuart, 1997).

Gloves can be tested with tracer gas during the test of the cabinet. However, gloves are a weak link in the integrity of the system and should be checked after intervals of service for pinhole leaks using the soap bubble test (NCI, 1979). The design of the cabinet should allow gloves to be changed without breaching containment using the "glove over glove" replacement procedure or other suitable method.

HEPA filter leak testing is required for certification of the integrity of the system. It requires no leak in the HEPA installation greater than 0.01% of the up stream concentration, performed following NSF International Standard #49 (NSF, 1992).

Negative pressure within the operating cabinet is demonstrated to be at least -0.5" w.c. using calibrated equipment (NCI, 1979). There should be a monitor calibrated to alarm if the pressure inside the cabinet goes positive of -0.5" w.c.

If needed, air velocities and air changes can be calculated from cfm values measured in the exhaust duct or at the intake port. Air flow patterns are checked in unidirectional down flow cabinets with smoke.

When the application requires a certain air cleanliness level, particle counts are measured with a single particle counter and the air cleanliness classification is calculated following Federal Standard 209E (Federal Supply Service, 1992).

Leak tightness of exhaust ducts is checked by releasing 1 oz. per 30 ft^3 R-12 into the sealed duct and scanning for a leak rate of no more than $1X10^{-4}$ cc/s (NCI, 1979). The cabinets have to be hard-connected to the building exhaust ducts in a way that will meet the requirements of this test.

Sterilizers are tested with *B. stearothermophilus* for steam and *B. subtilis* for ethylene oxide sterilizers by placing the spore strip in the fold of a towel within the load, running the cycle, aseptically placing the spore strip in a suitable broth medium and looking for no growth after seven days of incubation (NCI, 1979). Incinerators on the exhaust air systems are tested to show that all spores (*B. subtitlis*) are destroyed when the incinerator is challenged at a concentration of 105 spores per ft^3 of exhaust air.

Comfort tests such as lighting, noise and vibration can be performed, if needed, following NSF 49 (NSF, 1992).

A thorough method of validating the construction, installation, operation and performance specifications of a Class III system is to follow a detailed installation, operation and performance qualification (IQ/OQ/PQ) procedure. This documents that the required utilities and services are in place, that the components of the system are delivered as specified, and that the system conforms to the operation and performance requirements when tested using the accepted test protocols and instruments that are verified to be in calibration.

Certification of Biological Safety Cabinets must be performed by qualified personnel using calibrated instruments (Richmond, 1995). It is prudent to use certifying personnel who have experience with Class III systems.

Working Inside a Class III Biological Safety Cabinet

Because of the scarcity of such maximum containment laboratories, it is difficult for investigators to be trained beforehand for work in a Class III biosafety cabinet. For this reason, the laboratory director must restrict access to the laboratory and provide specific supervised training to new investigators. Students and casual visitors should not be permitted into the laboratory. Maintenance and repair workers who must enter the maximum containment laboratory must be escorted at all times by trained laboratorians.

Experience within a Class III cabinet maintained under maximum biocontainment room conditions consistently underscores to an investigator the need to carefully plan the workflow. The need for planning cannot be overstated for any experiment, even for the simplest procedures within the confines of a Class III Biological Safety Cabinet. Every item to be used in the experiment, including wipes, timers, biobags, dunk bath agents, etc., must be arranged at the beginning of an experiment, since any entry into the cabinet through the double-door autoclave must be followed by a decontamination cycle before opening the exterior door again. Each autoclave cycle is further extended in time since a cool down period is essential prior to reentry or exit. Every item for the performance of the experiment should go into the Class III cabinet on the first/last load. All too often however, one or two items are forgotten until the procedure has been started. This generally means an operator must discontinue the procedure, leave the maximum biocontainment laboratory, shower out, change, retrieve the item(s) needed, and go through the entry process from the beginning. Some of this down-time can be reduced through coordinated teamwork with support staff.

Without good training and even better planning, working within a maximum containment laboratory can be a discouragingly slow process even under the best of conditions. An investigator can move relatively easily between the Class III cabinet laboratory and adjacent work spaces, since suit decontamination does not have to be included in the exit process. Intervals between steps of an experiment in a cabinet laboratory can provide breaks for the investigator, whereas in the suit laboratory the individual generally cannot leave and re-enter without going through the elaborate suiting/de-suiting process.

Infectious material generated from the protocol generally is maintained in the Class III Biological Safety Cabinet, unless the product of the work are agent stocks. In this case, the agents can be safely removed from the Class III cabinet following decontamination of the carefully sealed non-breakable primary container (which has been placed into a non-breakable secondary container) by passage through a dunk tank containing an acceptable virucidal solution, e.g., cross-linked glutaraldehyde, suitably diluted bleach, or phenol solutions. These materials can then be stored in freezers located in the maximum containment laboratory.

All other materials removed from the Class III cabinet must be decontaminated by an autoclave cycle in the cabinet-attached autoclave equipped with interlocking doors. Since the autoclave opens into the surrounding maximum containment laboratory, a second decontamination in either the pass-through autoclave or fumigation chamber in the laboratory is required.

Final products of the experimental procedures are often materials other than virus stocks. In those cases, investigators have the options of i) working with infectious material within the cabinet, or ii) completely inactivating the materials for further work under BSL-2 conditions. Even though materials may have been inactivated in the Class III cabinet, containers must be decontaminated through a dunk tank or in some manner not destructive to the product to be used for final analysis. Decontaminated containers containing inactivated agents can then be exited from the room either by way of a second dunk tank or placed in a clean container and transported to a Class II Biological Safety Cabinet located in a BSL-2 environment for further work.

Laboratory environmental conditions are an important factor for the investigator(s). The temperature of the laboratory is a critical factor, since working either within a cabinet or a suit tends to generate a great deal of heat dissipation from the investigator. Work is strenuous under both conditions; however, within the cabinet environment, investigators have a great deal more latitude regarding their individual comfort zones. One is not dependent on suit air supply, nor the mini-environment of an impervious suit, since the cabinet is the primary biocontainment barrier. Generally, if the space is maintained at 65-68 degrees Fahrenheit, the work environment remains comfortable for each investigator throughout the experimental processes.

Personnel protection, including appropriate laboratory dress are generally part of the standard operating procedures for working in a Class III cabinet room at BSL-4 containment conditions. Surgical scrubs can be worn, and generally a surgical mask and eye protection are added to serve as barrier protection for the investigator. The mask and eye protection are optional; some researchers wear them as a reminder not to touch their face while in the lab. Since the pathogen will be maintained within the cabinet, there is no need to encase the investigator; instead, the agent is encased within the Class III cabinet. For BSL-4 work, the room is the secondary barrier for the department and institution which surround the specialized laboratory. The investigators take precautions over and above those described for work within a BSL-3 environment. After completion of work, scrubs are removed on the "dirty" side of the dressing facility, and the investigator showers prior to entering the clean side of the facility. Towels are provided on the clean side of the dressing area. Unisex dressing rooms are most practical in small facilities. Exit times can be staggered to accommodate one individual at a time.

Laundry from the clean side can be processed as general laboratory materials. Laundry from the dirty side is decontaminated in the pass-through room autoclave within the BSL-4 laboratory prior to removal from the facility for washing and recycling.

The overall procedures are time consuming and labor intensive in spite of detailed planning. However, once techniques are mastered and the investigator becomes familiar with working within this high containment laboratory, the advantages of using the

Class III Biological Safety Cabinet in a BSL-4 environment over a suit laboratory offer great economic savings (construction and maintenance) while still providing a safe and effective maximum containment laboratory. Additionally, maintenance and care of the unit, as well as the facility housing the unit can be much more effectively managed. Sections of the Class III cabinet can be closed and decontaminated for maintenance without closing the entire box, thereby permitting experiments to be continued year round.

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