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DESIGNING THE BSL4 LABORATORY (CHAPTER 9)

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ABSTRACT

This chapter will discuss the design issues of Biosafety Level 4 (BSL4) suit laboratories. It will review the requirements of "Biosafety in Microbiological and Biomedical Laboratories" (BMBL) (USDHHS, 1993), including revisions proposed for the 4th edition, in greater detail and give the rationale behind some of the requirements of laboratories at this level. The chapter will also detail issues related to layout and how this impacts operation with a comparative review of contemporary facilities.

INTRODUCTION

Biosafety Level 4 facilities have been romanticized in movies like "Outbreak" and books line the "Hot Zone"; however, these facilities are relatively straightforward. They utilize sound, proven engineering and operational principles to provide physical and operational barriers that keep personnel from contact with the infectious agents and allow safe operation. Due to these principles there have been minimal laboratory acquired infections during the history of their use, with minimal hazards to the communities in which they reside.

BMBL states that "Biosafety Level 4 laboratories are utilized for work with dangerous and exotic agents which pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route, and for which there is no available vaccine or therapy." The hazards of working with these agents as outlined in BMBL include autoinoculation, respiratory exposure to infectious aerosols, and mucous membrane exposure to infectious droplets. While facility design has an impact on the prevention of autoinoculation, it is a key factor in the prevention of exposure to infectious aerosols and droplets. Examples of agents used at BSL4 include viruses such as Marburg, Ebola and Congo-Crimean Hemorrhagic Fever.

There are two models for BSL4 laboratories intended for studying human disease; the "Cabinet Laboratory" where all handling of the agent is performed in a Class III Biological Safety Cabinet and the "Suit Laboratory" where personnel must wear a protective suit. BSL4 laboratories may be based on either model or a combination of both models in the same facility. This chapter will discuss suit laboratory design and maintenance issues.

BSL4 suit laboratories have been a relatively recent development. Major suit laboratories in the Western Hemisphere in long term use include those at the Centers for Disease Control and Prevention (CDC) in Atlanta and at the United States Army Medical Research in Infectious Disease (USAMRIID) facility in Ft. Detrick, Maryland. Within the last year, facilities have opened for Health Canada in Winnipeg, Canada (Chomiak, 1998 and Langevin, 1998), the National Institutes of Health (NIH), Bethesda, Maryland and the Merieaux Foundation (Fischer-Hoch, 1998), Lyon, France. Additional facilities are being contemplated worldwide including the Southwest Foundation for Biomedical Research (Kelley, 1998) in San Antonio, Texas. Other facilities that may provide insight into appropriate design are facilities developed for the study of exotic animal diseases such as the facilities at Geelong, Australia (Abraham, 1997).

Due to the small community utilizing BSL4 laboratories most of the information concerning the design of these laboratories has been passed "word of mouth." Recently the CDC took the initiative to bring together users and designers of BSL4 facilities into a "users group" that will allow a sharing of knowledge and ideas to make these facilities appropriate and effective for working with these agents.

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As with any biological laboratory there are three main concerns with design and operation:

- Protection of personnel working in the laboratory
- Protection of the specimens in the laboratory from contamination
- Protection of the environment outside of the laboratory

The suit laboratory uses pressurized suits as primary containment for personnel protection. These specially designed suits provide a physical barrier between the laboratory personnel and the organisms being studied.

Specimen protection is provided by use of Class II or Class III Biological Safety Cabinets (see chapter 3). These cabinets use directional airflow (class II), physical barriers (class III), and HEPA filtration to provide a clean environment for the samples. These cabinets also provide primary containment for the organisms being studied, minimizing organisms in the laboratory air. Proper procedures and equipment keep the air of a BSL4 laboratory free of organisms from routine procedures; however, accidental releases or releases during animal necropsies and similar procedures combined with the high level of hazard warrant the extreme precautions utilized in this type of lab to reduce the risk of exposure.

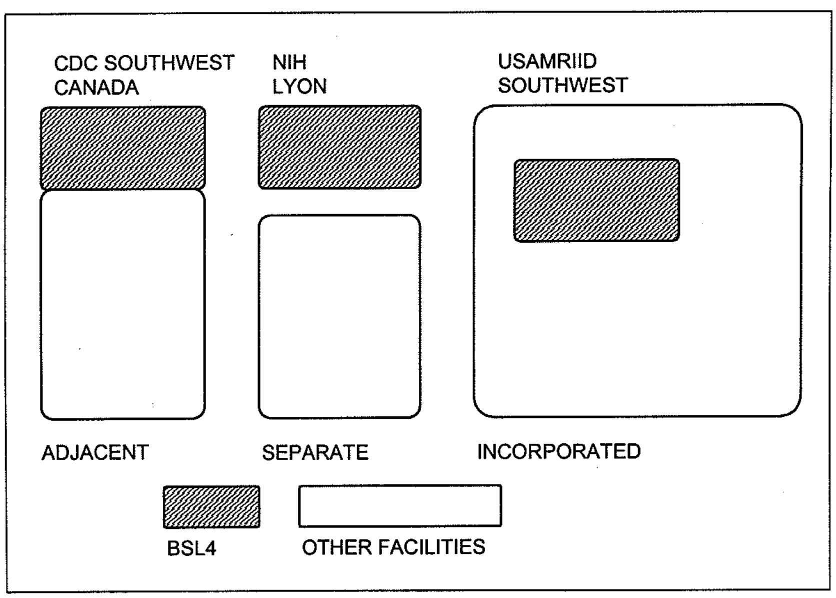
A physical barrier, directional airflow, HEPA-filtration of exhaust air and decontamination of materials, and waste when moving out of the facility are the means used to maintain environmental protection.

Location of the Laboratory Suite

The BSL4 laboratory is often a small component of the overall research infrastructure of the institution. BMBL requires that a Biosafety Level 4 facility be either a separate building or a clearly demarcated and isolated zone within a building. Most of the current and planned BSL4 facilities are adjacent to incorporated within a larger building. The BSL4 laboratories at CDC are separated by an atrium from the remainder of the facility, which is comprised of BSL2 and BSL3 laboratories support and related office space. The BSL4 lab is separated from other CDC facilities by a card access security system. The BSL4 laboratories and BSL4 clinical space at USAMRIID are located in the main building, separated by access control security systems. This building also houses space for routine clinical care of army personnel. The NIH BSL4 facility is a separate building that is not directly connected to the adjacent facility. From this operational experience, it appears that access control and system separation is more critical than location in a separate structure.

FIGURE 1

BSL4 Suite Location Models.



There are advantages to being located in the same building as the support facilities and lower level laboratories. This minimizes the requirements for movement of samples ad supplies. See figure 1 for a comparison of these models.

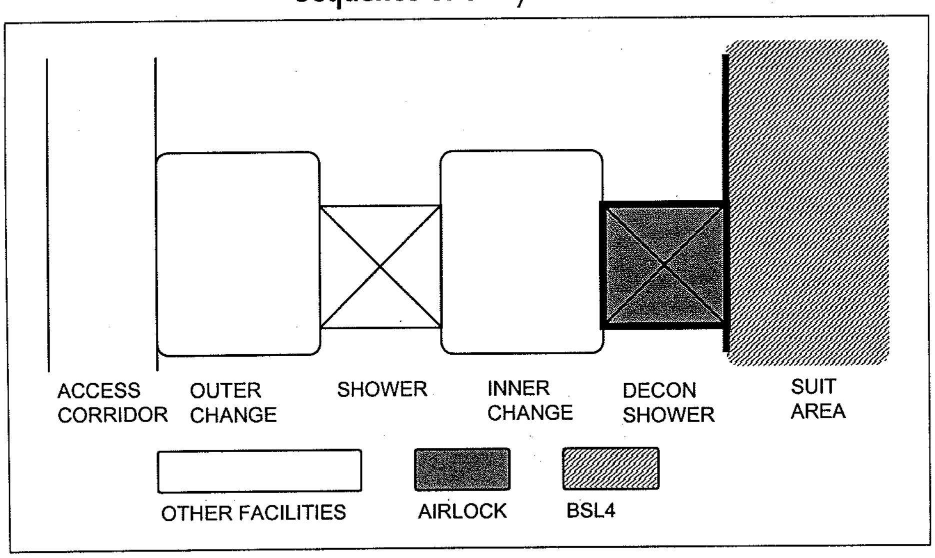
Entry and Exit of Personnel

Personnel enter the laboratory through a controlled access point. At NIH, because of the exterior access required as a separate building, an entry vestibule is provided. This vestibule doubles as a control room for the facility with visibility into the laboratory. This allows biosafety personnel to monitor the activities within the laboratory. Most of the other BSL4 suit laboratories are accessed directly from a secure corridor in the facility. At this point the user should be able to verify that all systems serving the laboratory are in proper modes of operation. A panel with fail/safe lights is generally provided. The entry access door to the laboratory must be self-closing and lockable. The laboratory is accessed through a series of rooms; generally the outer change room, shower, inner change room, decontamination shower. See figure 2. Passage through these areas is one person at a time. The decontamination shower serves as an airlock to keep a sealed airtight barrier in place at all times. At the Health Canada Laboratory the entry door and outer change room doors are also airtight providing additional barriers. This level of redundancy has not been shown to be necessary for safe operation.

The airlock doors are generally either pneumatic sealed doors or handwheel operated submarine style doors. The pneumatic doors are relatively easy to operate, but require more maintenance. Doors at CDC, NIH and USAMRIID are made from stainless steel and have pneumatic seals. The Health Canada lab has stainless steel doors with "handwheel operation." These doors, while generally maintenance free, are heavy and difficult to operate, particularly for less athletic laboratory personnel. The Lyon lab has pneumatic doors of solid plastic construction making them extremely easy to operate. If these doors withstand the operating and decontamination rigor of the BSL4 environment, they may prove to is a significant engineering improvement.

On passage in, the personnel remove clothes and other personal items. Secure locker space is necessary for storage of these items. Scrub suits or other appropriate clothing is put on. Personnel then step through the shower into the inner change room where the positive pressure suits are hung from racks. Each person is assigned an individual suit so adequate space must be provided for suit storage for all personnel working in the facility. An inspection table with good lighting should be provided to allow suits to be thoroughly inspected prior to wear. After donning the positive pressure suit, the investigator verifies the negative status of laboratory pressurization. The air pressure monitor must indicate directional airflow into the suit area, before the

FIGURE 2
Sequence of entry Rooms.



investigator opens the outer airlock door and steps into the decontamination shower.

Once the outer airlock door to the decontamination shower is closed the inner door can be opened. Once the inner door is opened, the airlock is potentially contaminated. An interlock is provided to prevent the outer airlock door from being opened until a decontamination cycle of the shower has been completed. An emergency release of this interlock to allow personnel to exit the laboratory in the event of failure should be provided. The investigator then enters the BSL4 laboratory adding boots for protection of the suit. A boot rack should be provided for storage just inside the entry to the suit area.

When exiting the suit area, the investigator removes the boots and places them on the rack and enters the decontamination shower. The decontamination shower cycle is then run to disinfect the outside of the suit. The disinfectant is typically Lysol or similar disinfectant pumped from a holding tank. Acceptability of the specific disinfectant in the local sewage system should be verified prior to its selection. The showerheads tend to clog. NIH has resolved this problem by providing "quick disconnect" shower heads that can easily be removed and cleaned. After the decontamination shower the investigator moves into the outer change room, removes the suit, takes a shower, redresses and exits the facility. These entry and exit rooms have generally been minimized on a square footage basis to save space; however, due to the low cost of these areas when compared to the cost of a BSL4 facility it would be wise to make these areas generously sized for user comfort and emergency situations that may arise requiring having additional personnel in the area. The facility at Southwest provides rooms that will be comfortable to use.

The Suit Area

The suit area is the space within the lab where all manipulations with infectious agents are done. The entire perimeter of the suit areas must be constructed to provide a sealed airtight shell to contain air within the facility in the event of a system failure. All penetrations into the internal shell of the suit area, chemical shower, and airlocks are sealed. This barrier also facilitates gas decontamination and prevents entry of animals and insects. The internal surfaces are selected to be resistant to the chemicals and fumigants used to decontaminate the area and to prevent spills from migrating into other areas.

The construction of this shell is usually concrete or concrete masonry units to provide heavy stiff construction. Both these materials are subject to shrinking and cracking over time and require routine inspection and maintenance. The Health Canada laboratory utilized specifically formulated concrete poured early in the schedule to ensure minimal shrinkage and cracking. CDC and USAMRIID utilize concrete masonry unit (CMU) construction. Experience has shown that CMU requires periodic maintenance to maintain complete airtightness. The Lyon laboratory is built straddling an existing laboratory building. Spanning this building required lightweight construction. Concrete walls were not practical. A system of steel faced urethane panels approximately 5" thick was utilized to form the barrier. These panels, normally used for the construction of environmental rooms, are joined with silicon sealant and cam action locks. This construction is fast, stable and cost effective.

There is not full agreement on the level of airtightness required in this shell. Some laboratories (USAMRIID, Lyon) do not pressure decay test the shell while others (Canada, Southwest) go through elaborate procedures to ensure a high degree of airtightness. Is the time and money required to create an absolute barrier necessary when the high differential directional air flow keeps contaminated air inside the facility? This is more debatable when you note that we purposefully pump air out of these facilities through double-banks of HEPA filters.

All services that pass through the shell must be protected with backflow prevention or filtration by HEPA filters. Any drains in the floors of the suit area contain traps that are filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer vents and other ventilation lines contain HEPA filters. Internal facility appurtenances in the suit area, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the exposed horizontal surface area.

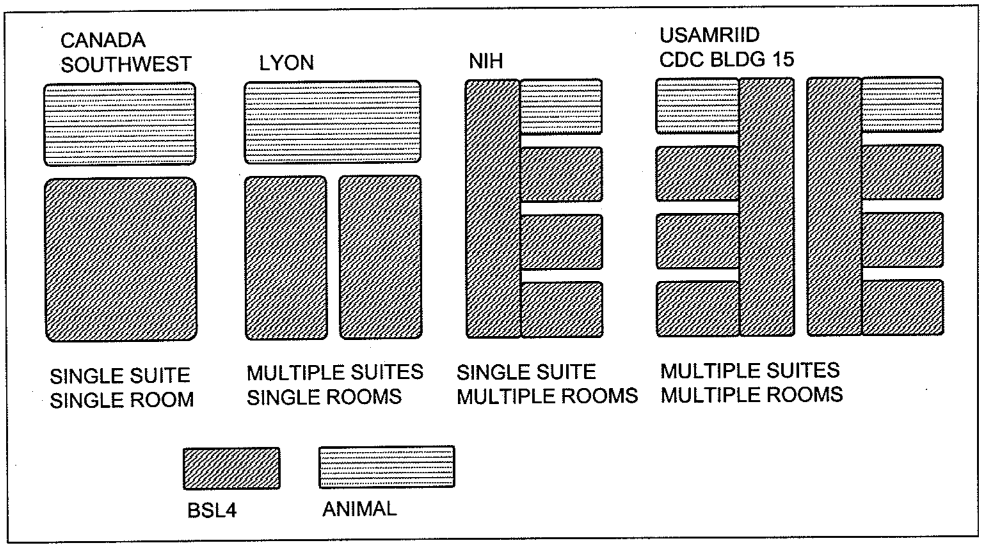
The room layouts of the suit areas vary widely. See figure 3 for examples of layouts. Single suite, single room layouts as planned in the facilities at

Southwest or a single room with adjacent animal holding as found at the Health Canada lab are the minimal scheme currently utilized. These models are the least flexible as only fully compatible operations can be performed at the same time. There is no redundancy provided when the lab is shut down for periodic maintenance. Other models such as the new facility at NIH have multiple rooms within a suite that can be used for different purposes. CDC and Lyon have multiple suites in their facilities that can operate independently. Each has adjoining independent animal areas. A suite can be shut down and the lab can still operate in the other suite. Also, two totally different programs can easily operate at the same time. The Lyon lab anticipates utilizing one suite for BSL3 work when not required for BSL4 use. CDC and Lyon plan regular alternating shutdowns of one f the suites for maintenance purposes. USAMRIID has multiple facilities, with multiple rooms, however they operate without shutdowns unless absolutely necessary.

The room uses inside a suit area generally include tissue culture and agent manipulation, equipment areas for centrifuges and other large equipment, sample storage, animal holding, necropsy, storage and decontamination preparation.

Windows to allow viewing into the BSL4 Laboratory and between areas of the labs should be considered. The windows can increase safety by providing visual access between personnel. The windows can be helpful when giving tours of the facility without having to expose people to the BSL4 environment. Any windows must use breakage resistant material.

FIGURE 3
Suit Area Models.



Furniture in the suit area should be of simple open construction to avoid concealed surfaces. NIH has provided all furniture on lockable casters to allow easy reconfiguration and cleaning. Easily removable cabinets are used at USAMRIID to allow flexibility in room usage. Bench tops should have seamless surfaces and should be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat including chemicals used to decontaminate the work surfaces and equipment.

Class II Biological Safety Cabinets, if used for primary containment, should be placed in the laboratory in a manner to allow them to operate within their specified performance envelope. As these cabinets are sensitive to environmental influences such as air velocities, door swings and personnel movements, proper room design can impact their performance. The HEPA filtered exhaust air from Class II Biological Safety Cabinets in a suit area may be recirculated back into the suit area.

Even though routine use is not anticipated, a foot, elbow, or automatically operated handwashing sink should be provided near the door of the suit area for emergency purposes and to facilitate servicing the suite.

Entry and Exit of Materials

Provisions for material movement both into and out of the suite is critical for ease of operation and to minimize the need for suit area shut downs. If equipment can be decontaminated and removed from the suit area for maintenance, laboratory operations can continue indefinitely. There are generally three methods for taking materials into and out of the facility. Small samples can be passed through a dunk tank or carried with the investigator through the entry shower of the laboratory. Medium size objects can be passed through a cool double-door, through-the-wall autoclave that has previously had its chamber decontaminated. Supply carts and other equipment can be brought in this way if a large roll-through autoclave is provided. This minimizes the need for paraformaldehyde gas decontamination of the airlock that is normally used for entry and removal of equipment too large to enter the facility through other means. Waste materials are decontaminated in the pass-through autoclave prior to being removed from the suit area. Autoclaves are sealed to the outer wall of the suit area to keep the sealed shell of the suit area intact. The autoclave is automatically controlled to make sure that the outside door can only be opened after the autoclave "sterilization" cycle has been run.

As space decontamination is routine in BSL4 laboratories, provisions should be made in the decontamination airlock to make this task simple. For paraformaldehyde decontamination, either ports for a paraformaldehyde generator and neutralizer or switched electrical outlets for frying pans should be provided. A wall fan to mix the air and remotely controlled dampers to isolate then exhaust the space should also be provided. The NIH facility is planning to utilize vapor phase hydrogen peroxide for its decontamination airlock and has installed a peroxide generator for this purpose.

Suits and Life Support System

The primary protection for personnel who enter the suit area is a one-piece positive pressure suit that is ventilated by a life support system. Care should be taken to ensure that the facility has no sharp edges or corners that could snag a suit causing a tear. The suit is protected by HEPA filtration at the air entry point to the suit. The suits may also contain radio headsets for enhanced communication capabilities. The suits that have been in use in the US are relatively heavy and noisy, making long hours somewhat uncomfortable. The Lyon lab is developing suits that are lighter in weight with systems that reduce noise and discomfort.

The suits are connected by a quick disconnect coupling to extendable breathing air lines that are placed in strategic locations throughout the facility. These locations should include the inner change room, decontamination shower and all work areas within the facility. A connection convenient to the suit inspection table in the inner change room should be provided to facilitate testing of the suits. While most current labs have airline coils dropped directly from the ceiling, the Geelong lab uses overhead coils on stainless steel cables to provide additional movement in the facility.

The air supply for this system is oil free breathing air provided by redundant air compressors and holding tanks. The air should be cooled and humidified, then piped into the laboratory to the required locations. Additional emergency capability is provided by backup breathing air tanks that are connected to the system to provide a minimum of 15 minutes of air for the personnel in the facility to shut down experiments and decontaminate them prior to leaving. Both audible and visual alarms should be activated when backup breathing air tanks are activated.

Mechanical Systems

The mechanical systems for the BSL4 facility play an important role in safety and are the most complex parts of the facility. They control the movement of fluids (including air) through and out of the facility. These fluids have potential for contamination and must be filtered or otherwise decontaminated prior to leaving the BSL4 laboratory. Supply lines must also be filtered or otherwise protected to prevent backflow of potentially contaminated materials.

The air supply and exhaust system for the BSL4 laboratory must not serve other areas of the facility. In a suit lab, this separate system would serve only areas within the sealed shell and airlocks with other systems serving support rooms and other clean areas of the facility. This minimizes the potential for contamination of clean areas. Directional airflow created by maintained (negative) pressure differentials is the method used to contain potentially contaminated air in the suit area. The directional airflow moves from areas of lesser hazard to areas of higher hazard. As specimens are always contained in normal operations in the laboratory areas of most BSL4 laboratories, the highest hazard areas are animal holding and necropsy rooms due to the aerosols generated in these areas that may be difficult to fully contain. Centrifuge rooms, due to the potential for a rotor failure to release high levels of infectious aerosols, should also be considered higher hazard.

No specific pressure differential is required by BMBL and setpoints vary widely between laboratories. Health Canada uses a series of four airlocks with a 50 Pascal (.2″ w.g.) differential pressure between each airlock. The NIH lab uses 50 Pascal (.2″ w.g.) at the shell of the facility then approximately 12 Pascal (.05″ w.g.) for differentials within the suit area and in the entry sequence. The Lyon lab is planning a 10 Pascal (.04″ w.g.) differential at each step with 30 Pascal (.12″ w.g.) between the exterior corridor and the suit area. The Geelong facility uses 100 (.4″ w.g.) Pascal increments with a 200 Pascal (.8″ w.g.) differential between the animal entry corridor and the suit area. The bottom line is providing a pressure differential that will create sufficient directional inward airflow. The lower differentials make opening and closing doors easier.

The air supply into the suit area, decontamination shower and decontamination airlock is HEPA filtered to prevent organisms from passing in the event of backflow. This filtration has the added advantage of providing a clean laboratory and extending the life of the exhaust HEPA filters. The exhaust air, from the suit area, decontamination shower and decontamination airlock, is filtered by two HEPA filters installed in series. A second set of filters with bypass dampers is provided for redundant operation. The facility design should allow the HEPA filters to be located as near as practicable to the suit area in order to minimize the length of potentially contaminated ductwork. Ideally, as at USAMRIID, the filters should be arranged to allow decontamination, removal and replacement of either filter bank without facility shutdown. The exhaust fan system should have multiple fans with full redundant operation. The exhaust fans should be located as close as practicable to the point of discharge from the facility. his discharge should be located away from intakes and other openings to minimize the potential for re-entrainment of the exhausted air into the building. Also, because the ductwork after the fans is pressurized. Ideally the fans should be located on the roof of the facility to eliminate circulation of the exhaust in the mechanical space.

Care must be taken in the design of the filter systems to ensure that the system conforms to the planned validation and decontamination protocol. The HEPA filter housings must be designed to allow for in-place decontamination of the filter prior to removal, or removal of the filter in a sealed gas-tight primary container for subsequent decontamination or destruction by incineration. The design of the HEPA filter housing should facilitate validation of the filter installation. Providing upstream ports for test substance introduction and downstream ports for probes to verify that the filter and seals are not leaking does this. Filter scanning modules should be considered for in place scanning of filters.

The supply and exhaust systems must be on emergency power and be interlocked to prevent the supply fan from running if the exhaust fans fail thus pressurizing the suit area. The operational status of these systems and the differential pressure between spaces is monitored and alarmed to indicate proper operation and to warn personnel of improper operation.

A central vacuum system, if required, must be dedicated to the facility. Vacuum lines must be HEPA filtered prior to leaving the laboratory. These in-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Devices that prevent backflow protect other liquid and gas services to the suit area. Service penetrations into the facility should be minimized to reduce sealing failures and to reduce filtration points. One point of entry into the suit area with distribution within the suit area is preferable.

Liquid effluents that potentially contain infectious agents, from sinks, floor drains, autoclave chambers and other sources within the suit area or airlocks must be decontaminated by a proven method. This has historically been done by heat treatment. The process used for decontamination of liquid wastes must be validated physically and biologically. The heat treatment is generally provided by taking the effluent into holding tanks and pumping it into cooking tanks that heat the effluent to the required temperature to deactivate the agent in question. The effluent is then cooled prior to being discharged into the sewer system serving the facility. The Health Canada facility has developed a rendering system to disinfect its infectious waste, as much of the waste is solids due to the large size of the animals held there.

Electrical Systems

Electrical systems for a BSL4 laboratory must include normal power as well as an automatically starting emergency power source. The emergency power should run the entire facility including all lighting, HVAC and decontamination systems in the event of a power outage. As with other systems, penetrations through the shell should be minimized. Where conduits penetrate the shell, the ends, including airspaces within the cable insulation should be sealed.

Lighting should be adequate with fixtures either internal or external to the shell. The Health Canada laboratory made the lens of the lights a component of the shell of the ceiling, allowing bulbs to be changed from the mechanical room above the suit area.

Communications, alarms and security systems should be adequate to control access, communicate between personnel in the space and outside of the space, and notify personnel of system malfunction. Closed circuit television to allow monitoring of the suite provides an additional level of safety in the event of emergency.

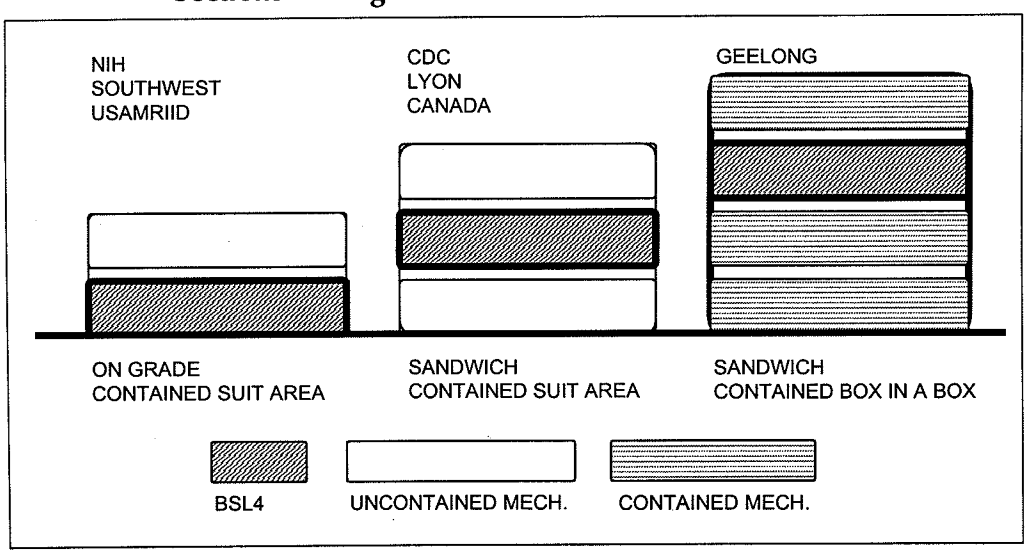
Overall Containment

Differing philosophies exist on how far to extend the containment shell. Most BSL4 facilities stop the containment shell at the ceiling, floor walls of the suit area and airlocks. Some facilities place the containment shell between two non-contained mechanical spaces to provide ease of access to services. The Geelong facility extends the containment shell around the primary mechanical rooms serving the facility creating a "box within a box." This places both the exhaust filters and the effluent decontamination tanks within a containment barrier. The major consideration for this is that the facility deals with exotic animal diseases that may have devastating effects if released in the environment. Figure 4 compares these models.

Commissioning and Verification

The completed Biosafety Level 4 facility must be tested for verification that the design and operational parameters have been met prior to operation. A specific plan should be developed that allows monitoring of the requirements throughout con-

FIGURE 4
Sections Through Mechanical Containment Models.



struction. Sufficient time should be allowed to ensure that conditions are met and minor modifications can be made. Training on operational and maintenance procedures should occur at this time.

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