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EDITOR'S PAGE

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Design of BSL3 Laboratories (Chapter 7) — Jonathan T. Crane and James F. Riley

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Role of the Class III Biological Safety Cabinet in Achieving Biological Safety Level 4 Containment (Chapter 10) — David G. Stuart, Julia Hilliard, Richard Henkel, Jack Kelley, and Jonathan Y. Richmond

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VISION

ABSA, the leader in the profession of biological safety.

MISSION STATEMENT

The American Biological Safety Association is dedicated to expanding biological safety awareness to prevent adverse occupational and environmental impact from biohazards.

GOALS

- Expand professional and public awareness of biological safety through effective communication.
- Participate in the development of biological safety standards, guidelines, and regulations.
- Develop ABSA as the recognized resource for profession and scientific expertise in biological safety.
- Advance biological safety as a scientific discipline through education, research, and professional development.
- Develop and maintain standards for biological safety professionals.
Every year when ABSA membership renewals arrive we acknowledge, on some level, that we are a member of a national biological safety organization which, hopefully, has an impact on us and on our jobs. But how important is this membership to us? When the dues are paid by the employer, I think we feel less ownership of that membership than if we had to pay for it out of our own pocket. However, the future accomplishments and activities of ABSA depends on the membership’s feeling of ownership, and it is this degree of involvement that is needed to have the greatest impact on our organization.

Putting ideas into practice requires a concerted effort between the members, the Council and the committee chairs. When we hired a management company in 1992, our involvement became less intense, especially for the ones planning the conference, but we still require members to be involved. There are many of the members who are active and work diligently to improve the organization, but we could do so much more if more members would get involved. The more people contributing, the more the possibilities.

In our jobs we are focused and committed to every project until it is completed, and then we stay involved as the program dictates. The same principle applies to members working on projects for ABSA. The success of our Organization is analogous to our success as a biological safety professional.

The best ways in which we can contribute are:

1. **Participating in a committee.** Being on the committee list doesn’t count. We have to participate. When a task or project is presented, it is our responsibility to respond as requested.

2. **Contributing articles to the Journal.** I hear people say they plan to submit an article or they have already determined that the subject matter is not interesting or within the mandate of the Journal’s mission. The subject matter for the Journal has been expanded by the new Journal editor to include all biosafety related topics. So please submit your articles.

3. **Serving as an officer.** The Nominating Committee selects candidates based on their previous contributions to ABSA. It is an honor to be recognized by the membership as being thought to be capable of contributing to the future of ABSA. I encourage you to accept the nomination when asked.

We also need to be involved in our affiliate organizations. Affiliates are able to provide current information, sometimes more efficiently than the national organization, because they usually meet quarterly, whereas the national organization meets only once a year. The affiliates also play a vital role in disseminating pertinent information, such as federal regulations, to their community members whose jobs may have only a small focus in biological safety, but need the information just as much as the biological safety professional.

Commitment of service at the local community level can be just as important as at the national level. The national organization and the affiliates are important to each other, and although we could function independently, we would be narrowing our scope of available information and service.

Active involvement of the membership is the cornerstone of a strong, healthy organization, and it takes more than just a few people. Get involved!

Marilyn Misenhimer, RBP, CBS
University of Southern California
Los Angeles, California
What distinguishes a biological safety professional from a non-professional? One important characteristic is that the professional knows where to find and use technical resources on biological safety. Members of ABSA are kept well informed by the Newsletter and Journal, use the ABSA website, and on their office bookshelves they have available the most relevant biosafety articles and books. First and foremost among the books, of course, is the CDC/NIH guidelines *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) the 4th edition of which is being printed as this article is being written.

Three years ago the ABSA Council and the Long Range Planning Committee under the leadership of President Joseph Van Houten developed the ABSA Strategic Plan. One goal of that plan was to develop a number of publications for ABSA. The Publications Committee, chaired by Jonathan Y. Richmond, was established to develop the first publications. A year ago our first publication, the *ABSA/CDC 5th National Symposium on Biosafety: Rational Basis for Biocontainment Proceedings*, edited by Jonathan Y. Richmond, rolled off the printing presses. It is still available from ABSA. This year our second publication *Anthology of Biosafety I: Perspectives on Laboratory Design* is now available. In this current issue of our journal we have reproduced four articles from this anthology on the popular subject of designing BSL-3 and 4 laboratories and the use of primary containment devices. The third publication *Anthology of Biosafety II: The Application of Principles* is well into development. After the 6th National Symposium “Prudent Practices for the New Millenium” which is scheduled for February of 2000 we hope to have our fourth publication, which would be the Proceedings of this symposium.

The articles in these publications are written by well known experts who share with us with their insight and detailed knowledge in a wide variety of biological safety subjects. They offer each of us the opportunity to expand our knowledge base in biological safety and they provide us with relevant and timely information which should enhance our skills and professionalism. Hopefully, each of our new publications will join the BMBL on our biological safety professional’s bookshelves.

These publications are the result of the combined efforts of the members of the Council, the Long Range Planning Committee, the authors, and the many ABSA members who have contributed to them. In particular, we must recognize Jonathan Y. Richmond whose central role in the development and production of each publication has enriched us all.

Richard C. Knudsen
Centers for Disease Control and Prevention
Atlanta, Georgia
INTRODUCTION TO THE JABSA EDITION ON CONTAINMENT DESIGN

Jonathan Y. Richmond
Centers for Disease Control and Prevention, Atlanta, Georgia

There has been a tremendous jump in interest in laboratory design for working with microbial agents during the past five years. This includes new facilities as well as renovations to improve our collective aging infrastructures.

ABSA has recognized this need and recently authorized the development of Anthology of Biosafety I: Perspectives on Laboratory Design, which was published earlier this year. Four papers have been chosen for inclusion in this volume of JABSA as a service to our members who have responsibilities as core team members in the review of plans or who otherwise contribute their expertise in assuring that appropriate primary and secondary biocontainment needs are met.

There are three additional publications that should be helpful to you, the biosafety professional, and to architects and engineers to design and build our laboratories:


Many ABSA members are contributing authors to these important publications; stay tuned for additional ABSA-sponsored materials in the near future.
PRIMARY CONTAINMENT (CHAPTER 3)

David G. Stuart
The Baker Company, Sanford, Maine

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 airflow 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.
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
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 6**
Example of Cabinet modification for a specific application.
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.
(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 it’s 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.

REFERENCES


DESIGN OF BSL3 LABORATORIES (CHAPTER 7)

Jonathan T. Crane and James F. Riley
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ABSTRACT

This chapter will provide design and layout information for Biosafety Level 3 (BSL3) laboratories showing how the guidelines translate into three dimensional architectural facilities. The basic models of the BLS3 laboratory will be presented with diagrams:

- BSL3 laboratory with anteroom or workroom as access zone
- BSL3 laboratory with restricted corridor as access zone
- BSL3 laboratory with BSL2 laboratory as access zone
- BSL3 laboratory suite

INTRODUCTION

The main issue regarding the layout of BSL3 laboratories in Biosafety in Microbiological and Biomedical Laboratories (BMBL) (DHHS, 1993) deals with access control by passage through two doors in series. This requirement has influence on how the BSL3 facility is designed and the laboratory workflow. As indicated in the Laboratory Safety Monograph, (DHHS, 1979) there have been a limited number of models utilized for laying out these facilities; however, there are many permutations of these models. The first three models are similar in nature. The difference between these models is the type of space one uses as the access zone to the BSL3 areas; an anteroom, a corridor or a laboratory. Each model should be used based on the program to be housed and it’s specific requirements. Room size and equipment layout is also critical to BSL3 laboratory operation.

BASIC MODELS

BSL3 Laboratory with Anteroom or Workroom as an Access Zone

The simplest BSL3 facility is a two-space facility with an entry door from an access corridor into an anteroom or workroom that serves as an access zone for the BSL3 portions of the facility. This anteroom can serve for clothes changing, supply storage and other functions that support the work in the BSL3 module. Work with hazardous agents that require BSL3 containment must be handled in the BSL3 space accessed through a second door. With single room facilities the anteroom is generally small in size, as there are limited functions that can take place in this area. A variation on the anteroom is to enlarge it into workroom. This allows preparation activity to take place near but outside of the BSL3 labs. As shown in Figure 1 the anteroom can be used to serve single or multiple BSL3 spaces.

This model of a BSL3 facility generally does not have a dedicated autoclave within it, usually due to cost and space limitations. The use is limited because functions such as centrifugation of live agents must occur in the BSL3 room with other functions such as tissue culture. However, storage of frozen agents in the anteroom has been found to be acceptable by many organizations. Figure 2 illustrates a typical layout.

Although it has limited use, this model of a BSL3 facility is popular due to the low cost of construction and the ease of using it for a BSL2 tissue culture when not in use at BSL3. It is generally found where small-scale sporadic BSL3 work is anticipated.

BSL3 Laboratory with Restricted Corridor as Access Zone

A second model provides access to BSL3 spaces directly from a corridor that has been closed to

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through traffic. The first door is encountered when entering the corridor and the BSL3 space begins when entering the rooms off the corridor.

This model has the same disadvantages as the prior one. Depending on the size of the program, an autoclave may be provided within the facility to decontaminate waste. This model is often used when there are a large number of small separate BSL3 programs. This model should be considered when converting existing laboratory space to BSL3, as often this concept will reduce the amount of construction required.

BSL3 Laboratory with BSL2 Laboratory as Access Zone

A modification to the above models is to enlarge the anteroom into a working BSL2 laboratory and provide small BSL3 modules off of the lab.

This model works well for isolation rooms and small functional laboratories in clinical laboratory
settings. Again, as in the initial model, due to the requirement that all BSL3 functions occur in the BSL3 space, this concept is limited to smaller scale BSL3 operations.

The BSL3 Laboratory Suite
More complex BSL3 laboratories work better in a suite concept that creates larger and more diverse spaces at BSL3. An anteroom leads into the suite with a BSL3 workroom. This workroom function as a preparation area that allows the modules to function effectively for dedicated purposes such as tissue culture. Additional rooms can be dedicated for equipment use, for centrifuges freeze dryers and other common equipment allowing shared use of this equipment.
This segregation can increase safety and effectiveness by creating good workflow in the BSL3 laboratory. This size facility often includes pass through autoclaves to allow decontamination to take place prior to hazards leaving the BSL3 facility.

This larger suite concept works well for more complex multi-functional BSL3 areas such as those used for large volume TB testing and identification, and large research programs dedicated to studying BSL3 agents.

**Design Issues for BSL3 Modules**

The basic space in a BSL3 facility is the module where the infectious agents are cultured and manipulated. These modules are usually sized to accommodate comfortable work in one or two biosafety levels.
cabinets in the same room. Sizes of the modules generally range from 8 x 10 feet for a single biosafety cabinet to 11 x 20 feet for two biosafety cabinets. The layout of the module should facilitate working in a biological safety cabinet by placing storage shelving, incubators and other relate equipment as close to the cabinets as possible. BSL3 modules usually have less casework than other laboratories with plenty of floor space for large equipment. Doors into the BSL3 facility should be sized to allow easy access for moving equipment such as biosafety cabinet and freezers.

The layout of the modules should place the biological safety cabinets away from the doors and walking paths to keep the cabinets operating properly. Figure 7 shows the layout of a small BSL3 module with the biological safety cabinet placed to minimized performance disruptions (Crane, 1994). The biosafety cabinet and the room size must be closely coordinated to ensure that the door swing does not suck or push air into the cabinet causing loss of containment. In a small room the preferred location is on a side wall.

If two cabinets are place in a single room the preferable location is face to face (possibly with a 24 to 36 inch offset) at the rear of the room as this location is the least disruptive to cabinet performance. If the cabinets are side by side, a person walking to the second cabinet will affect the containment capability of the cabinet closest to the door.

BSL3 modules usually have partial walls of benches with a sink for handwashing. For tissue culture rooms microscopy tables are often provided for checking specimens. Space for incubators, refrigerators and freezers should also be provided.

**FIGURE 7**

Typical Small Module with Single Biological Safety Cabinet.

![Diagram of typical small module with single biological safety cabinet]

**Design Issues for Associated BSL3 Spaces**

Entry and gowning areas, if provided, should be developed to allow for easily following the entry and exit protocols for the laboratory. Considerations should include lockers for personnel effects, shelving for gloves, gowns, masks and booties or jump suits if these items are required. Waste receptacles for removed protective clothing should be provided. A sink should be considered in this area. The entry gowning area is also a good space to store spill control and decontamination equipment and supplies.

BSL3 modules are often used for tissue culture functions. As there are numerous preparatory and related uses in a biomedical laboratory, common work areas outside of the BSL3 modules should be considered to house these functions.

Separate rooms for equipment support and storage should be considered. Centrifuges and other
large equipment can be easily shared if placed in an equipment room rather than a BSL3 module. The separation of this equipment can reduce hazard by isolation in the event of an accident such as a centrifuge rotor failure. The heat load from equipment such as low temperature freezers is more easily handled if grouped together where additional focused cooling such as fan coil units can be applied.

Although decontamination autoclaves are not required to be located within BSL3 laboratories, laboratories with significant amounts of hazardous waste should consider providing pass through autoclaves to decontaminate materials leaving the facility. The intent of this is to reduce the volume of infectious waste passing through corridors. Adequate holding space for the waste prior to decontamination is necessary to reduce potential for exposure to infectious agents. Consideration should be given to providing a service closet for the autoclave, as shown in Figure 6, to minimize the heat from the outside of the chamber from entering the work area. Access to this service area should be from outside the BSL3 facility to allow service to take place without exposing the service personnel to the BSL3 environment. Exhaust ventilation should be provided above the exterior door of the autoclave to remove heat and steam when the door is opened. The area outside the BSL3 suite where the autoclave discharges can also be used for gas cylinder storage to keep delivery personnel from having to enter the BSL3 area.

Animal holding space may be incorporated in BSL3 facilities. Care should be taken to provide appropriate containment equipment to keep aerosols generated by animal care from entering the general room environment. BSL3 animal facilities often contain personnel showers and change rooms to increase environmental protection. Figure 9 shows an example of a BSL3 animal suite with decontamination facilities.

Enhanced BSL3 Laboratories

In some instances, it may be necessary to add facility features not required by the BMBL guidelines. Generally, this is increased environmental protection in the form of HEPA filtration of the exhaust air, effluent decontamination, or the addition of a personnel shower in the changing area.

CONCLUSION

Programmatic issues drive the layout of BSL3 facilities. The current BMBL facility guidelines have minimal impact on the layout. The model chosen for a specific facility must be based on the intended scope of operations for the facility to be successful.
REFERENCES


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 like 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 Merieux 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.

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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 and 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 air-tight 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 of 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.

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 BML 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.

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<th>CDC</th>
<th>GEELONG</th>
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<th>ON GRADE CONTAINED SUIT AREA</th>
<th>SANDWICH CONTAINED SUIT AREA</th>
<th>SANDWICH CONTAINED BOX IN A BOX</th>
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<td>BSL4</td>
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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.

REFERENCES


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 prevent 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 biocountainment 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-urder-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 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.

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 ft³ 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 4.5X10⁶ cc/s. In 1976 the Federal Register published the requirement as “leak rate <1 by 10⁶ 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 1X10⁸ 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 ft³ of cabinet volume, pressurizing to 3” w.c. with air and alarming at 1X10⁷ 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 3X10⁷ 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 ft³ 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. subtilis) are destroyed when the incinerator is challenged at a concentration of 105 spores per ft³ 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 laboratory workers.

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. Urine 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.

REFERENCES


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