1
Laboratory Biosafety in Containment Laboratories

Annette A. Kraus and Ali Mirazimi

Microbiological research on and diagnostics of highly pathogenic microorganisms, namely bacteria and viruses, have to be conducted in containment laboratories in order to contain the infectious material. We are therefore referring to the ''Concept of Biocontainment'' – a concept, which dates back to the 1940s, when the first biosafety cabinet (BSC) class III was developed at the US Army Biological Warfare Laboratories in Fort Detrick, Maryland.

Biocontainment is required to prevent accidental infection of researchers or diagnostic staff and to avoid release of the infectious agents into the surrounding environment.

1.1
Routes of Infection

Depending on the nature of the microorganism, there is a great variety of infection routes. However, the natural route of transmission may be different in a laboratory setting when working with isolated pathogens. This has to be considered when establishing working procedures for biosafety laboratories.

In a research laboratory, for instance, HIV or hepatitis B virus is not transmitted via the natural route, that is, from person to person through direct contact of body fluids, but, for example, through accidental inoculation with a syringe. The bacterial pathogen *Neisseria gonorrhoeae* spreads through direct contact under normal circumstances and laboratory workers have also to primarily protect themselves from direct contact (Chapter 10).

Bacteria and viruses that are vector-borne such as the tick-borne encephalitis virus (TBE) or the tick-borne *Borrelia* sp. obviously have a different infection route in the laboratory as compared to the natural setting. Here, protection should also aim at preventing needlestick injuries with contaminated syringes and direct contact with fluids that have a high concentration of the infectious agent.

While some other bacteria such as *Salmonella* or *Vibrio cholerae* spread via the fecal-oral route through contaminated food or water and are relatively easy to contain, others spread readily via aerosols and are more difficult to control.

Working in Biosafety Level 3 and 4 Laboratories: A Practical Introduction, First Edition.
Edited by Manfred Weidmann, Nigel Silman, Patrick Butaye, and Mandy Elschner.

Consequently, when working with bacteria such as *Mycobacterium tuberculosis*, laboratory workers have to protect themselves by wearing personal protection devices (respirators) and perform the work in BSCs. The same measures have to be taken when working with viral pathogens that also spread through aerosols, such as the avian influenza virus or hantaviruses.

In general, airborne or aerosol-transmitted pathogens are comparatively difficult to work with. Aerosols are practically invisible to the human eye, not to mention airborne viruses or bacteria. Using syringes, pipettes, and mixing devices, even according to good laboratory practice (GLP) protocols, creates aerosols in unexpected amounts. Dimmick *et al*. conducted a study in the early 1970s and estimated the aerosol dose originating from pipetting 1 ml to be 10^{10} small particles. Even at a distance of 3 m, the number of small particles still reaches 50. Depending on the nature of the pathogen, this can clearly reach or exceed the infectious dose of that particular virus or bacterium.

Generally, different precautionary measures have to be applied when working with different pathogens according to the routes of transmission.

1.2 Classification of Microorganisms

When establishing routines for microbiological laboratories, not only the route of infection of the used pathogens has to be considered, but the infectious dose, available countermeasures, and preexisting immunity have to be considered as well. In addition, information about concentration of the isolated pathogen, total volumes used in a certain research or diagnostic setting, as well as experience is important when defining the risk.

While airborne viruses belong to the most difficult pathogens to contain, work with varicella virus, for instance, can be performed under moderate safety precautions, because the disease is treatable and a vaccine is available. HIV, on the other hand, causes a lethal disease that can be neither treated nor prevented by a vaccine, is a rather unstable virus with a comparatively low infectious dose, and can therefore also be handled with moderate safety precautions.

The obvious question is now to classify the microorganisms and translate this classification into levels of precautionary measures, that is, into biological safety levels (BSLs). The levels of containment range from the lowest safety level 1 to the highest at level 4. In the United States, the Centers for Disease Control and Prevention (CDC) have specified these levels. In the European Union, the same BSLs are defined in a directive (Commission Directive 97/65/EC). In summary, the classification of microorganisms is based on various parameters specific for every pathogen including routes of transmission, severity of the disease, infectious dose, available countermeasures or preventive measures, and transmissibility to the community. These may be influenced by existing levels of immunity, density and movement of host population presence of appropriate vectors, and standards of environmental hygiene.

The WHO (World Health Organization) has classified infectious microorganisms by risk groups and the following list provides an overview of the risk levels when working with different pathogens. There are however other classification schemes (Chapter 2):

WHO risk group 1 – minimal risk
Microorganisms that usually do not cause human disease, such as *Escherichia coli* K12 or *Lactobacillus*.

WHO risk group 2 – moderate risk
Microorganisms that cause treatable or self-healing diseases and are difficult to contract via aerosol in a laboratory setting, such as salmonella or measles virus.

WHO risk group 3 – high risk
Highly contagious microorganisms that cause serious diseases, such as TBE virus or *M. tuberculosis*.

WHO risk group 4 – very high risk
Highly contagious microorganisms that cause serious diseases, even epidemics, with high mortality rate, such as Ebola virus or Lassa fever virus.

1.3 General Containment Principles

In general, there are two different levels of protection against accidental infections when working with pathogens in a research or diagnostic laboratory, a primary and a secondary barrier. In addition to these, safe working procedures and techniques together with safety equipment complement a containment laboratory.

Primary containment provides the protection of personnel and the immediate laboratory environment from exposure to infectious agents and is provided by good microbiological technique and the use of appropriate safety equipment, such as BSC. Secondary containment is the protection of the environment external to the laboratory from exposure to infectious materials and is provided by a combination of facility design and operational practices.

1.4 Specific Containment Principles

At the lowest level of biocontainment, the containment zone may only be a chemical fume hood. At the highest level, the containment involves isolation of the organism by means of building systems, sealed rooms, sealed containers, personal protective equipment, and detailed procedures for entering the laboratories, coupled with decontamination procedures when leaving them. In most cases, this also includes

high levels of security for access to the facility, ensuring that only authorized personnel may be admitted to such laboratories.

The following list describes the different specific measures of the BSLs 1–4 laboratories.

1.4.1 Biosafety Level 1 Laboratory

This level is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adults and of minimal potential hazard to laboratory personnel and the environment (CDC, 1997). At this level, precautions against the biohazardous materials in question are minimal, most likely involving gloves and perhaps some sort of facial protection (if indicated by risk assessment). Decontamination procedures for this level are similar in most respects to modern precautions against everyday microorganisms, that is, washing hands with antibacterial soap and/or washing all exposed surfaces of the laboratory with disinfectants.

1.4.2 Biosafety Level 2 Laboratory

This level is similar to BSL-1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. Here, laboratory staff have specific training in handling pathogenic agents, and consequently, access to the laboratory is restricted. Extreme precautions are taken with contaminated syringes and other sharp items to avoid accidental infections. In addition, certain procedures in which infectious aerosols or splashes may be created are conducted in a BSC.

1.4.3 Biosafety Level 3 Laboratory

This level is applicable to research and diagnostic laboratories in which work is done with indigenous or exotic agents, which may cause serious or potentially lethal disease on transmission. Laboratory staff have specific training in handling pathogenic agents. All procedures involving the manipulation of infectious materials are conducted within a BSC, or other physical containment devices (engineering controls), and by personnel wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features, so that, for instance, the filtered exhaust air from the laboratory room is discharged to the outside. In addition, the laboratory is negatively pressurized and entry and exit are therefore limited through an air lock. Access to the laboratory is restricted when work is in progress, and the recommended standard microbiological practices and safety procedures for BSL-3 are rigorously followed. Basically however a BSL-3 laboratory is designed to protect the environment from contamination by pathogens.

It does not increase safety for laboratory staff; this is the function of the primary engineering controls employed.

1.4.4 Biosafety Level 4 Laboratory

Dealing with biological hazards at this level requires either suit-based or cabinet-line-based BSL-4 laboratories. As there are only a few biocontainment laboratories that use class III biological safety cabinets, this chapter focuses on the use of a one-piece positive pressure personnel suit in combination with a class II biological safety cabinet and a self-contained breathing-air supply in a high-containment laboratory with negative pressure.

1.5 Design of a Suit-Based-BSL-4 Laboratory with Negative Pressure

Building a BSL-4 laboratory is a huge technical undertaking. For every square meter of laboratory space, 5 m^2 of technical area is required. In principle, all that is needed is a room with a BSC. This room however has to be completely isolated from other rooms in the building, and ideally, the facility is either in a separate building or in a controlled area, which is completely isolated from all other areas of the building.

Rooms in the facility must be arranged to ensure exit by sequential passage through the chemical shower, inner (dirty) suit room, personal shower, and outer (clean) changing area. An air lock fitted with airtight doors is used during personnel entry and exit. To enter and move material to and from the laboratory, electronically secured air locks are employed to prevent both doors being opened at the same time.

Safe entry and exit procedures have to be established for the laboratory staff. The chemical shower is used to decontaminate the surface of the positive pressure suit before the worker leaves the laboratory. In addition, there must be a system to take materials of various natures, ranging from heavy technical equipment to highly sensitive samples, into the laboratory and potentially out again. This could be a fumigation chamber or a dunk tank filled with a decontaminant.

The BSL-4 laboratory as a sealed entity has special engineering and design features to prevent microorganisms from being disseminated into the environment.

To this aim, the laboratory is kept at negative air pressure, so that air flows into the room if the barrier is penetrated or breached. The supply and exhaust components of the filtered ventilation system must be designed to guarantee a set rate of air changes, to maintain the negative pressure to surrounding areas, and to provide differential pressure or directional airflow as appropriate between adjacent areas within the laboratory. Several concepts of BSL-4 laboratory structure have been designed. Sandwich type laboratories house the laboratory floor in between technical floors generally providing supplies from above and treating

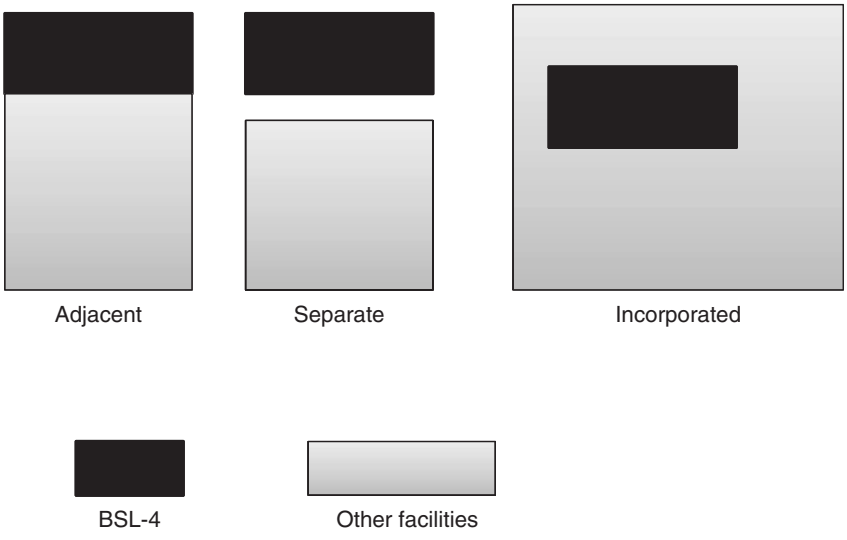

Figure 1.1 BSL-4 suit models.

waste and effluents below. The technical and laboratory sections can however also be arranged adjacent to each other or the laboratory space is arranged inside the shell of the technical section in a box-in-a-box containment approach [1] (Figure 1.1).

All work in the level 4 laboratory is done in a pressurized and ventilated suit. Air for breathing is passed into the suit through a hose and is filtered to be free of microorganisms. In addition, most activities involving pathogens in the work areas of the facility are confined to a class II BSC. In contrast to a BSL-3 laboratory, a BSL-4 laboratory therefore provides maximum protection for laboratory staff as well as preventing release of pathogens to the environment.

To perform laboratory work, access to water and obviously to a sewer system is necessary. All air and water services coming from a BSL-4 laboratory will undergo decontamination procedures to eliminate the possibility of an accidental release. Waste will be decontaminated through double-door autoclaves.

Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to chemicals used for cleaning and decontamination of the area.

For safety as well as practical reasons, communication devices have to be installed providing telephone, fax, and data lines. Research and diagnostic laboratory members as well as technical staff have to be trained in handling hazardous infectious agents. They must understand the primary and secondary containment functions of the standard and special practices, the containment equipment, and the laboratory design characteristics. Finally, all work done in the laboratory needs to be documented and access to the laboratory is strictly controlled.

1.6 Safety Routines

In order to maintain a high level of safety as well as security in a biosafety laboratory, certain rules and routines have to be established and strictly enforced. Even though the regulations will vary to a certain extent in different laboratories – being situated in different countries with specific directives, handling particular specimens or based on specific physical premises – the regulations are in principle the same.

Primarily, access to the containment laboratory is restricted to authorized personnel only (who has passed a designed course for high containment laboratory level 4). Research and diagnostic staff are thoroughly checked and trained before being granted access to the laboratory. The training includes several working hours under supervision of a mentor with experience in the biosafety laboratory. Annual exercises of the safety routines are mandatory and ensure a high standard of preparedness for the case of an emergency.

The daily routines for working in the BSL-4 laboratory are based on a ''buddy system'' or camera-based system and include a list of checkpoints. There should also be a decontamination team available outside of the laboratory, which provides trained help in case of an emergency.

Before the BSL-4 laboratory can be entered and work can commence, a list of key points has to be checked by the research or diagnostic personnel. A number of technical parameters will be controlled every time, such as the pressure status of the laboratory and the adjacent rooms, or whether the internal communication system is working. In addition, the filling status of the chemical shower supply tanks is checked as well as the air supply and ventilation systems. The actual access to the laboratory is then restricted to authorized staff. Once inside the laboratory wing and changing rooms, researchers change into common laboratory clothing and activate the personal communication devices. The overpressure safety suits will be put on and connected to the air supply in the so-called suit room. Finally, the researchers gain access to the actual work place through an air lock. A recommended working period of 3–5 h should not be exceeded. Exit from the laboratory is essentially the reverse process with the addition of a suit decontamination step in the chemical shower.

Summary

Outbreaks of emerging infectious diseases continue to challenge both human and veterinary health in Europe and around the world. Events such as the outbreaks of SARS-CoV (severe acute respiratory syndrome coronavirus), H5N1 avian influenza, Ebola virus in the Congo, Lassa fever in West Africa, and Crimean–Congo hemorrhagic fever virus (CCHFV) in Europe. To develop strategies to prevent outbreaks and combat these diseases, we need to develop research and diagnostic platforms. Research and diagnostics of highly pathogenic microorganism have to be conducted in containment laboratories

in order to contain the infectious material. The WHO has classified infectious microorganisms by risk groups from 1 to 4. In this chapter, we discuss the biocontainment that can be used to handle risk class IV pathogens. Dealing with biological hazards at this level requires either suit-based or cabinet-line-based BSL-4 laboratories. In this chapter, we focus on the use of a one-piece positive pressure personnel suit in combination with a class II biological safety cabinet and a self-contained breathing-air supply in a high-containment laboratory with negative pressure.

Reference

1. Crane, C.T., Bullock, C.F., and Richmond, J.Y. (1999) Designing the BSL4 laboratory (chapter 9). *J. Am. Biol. Saf. Assoc.*, **4** (1), 24–32.