



# Implementation of a Biosafety Level 3 (BSL-3) Facility in Singapore: Requirements, Work Practices, and Procedures

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## Abstract

*The Nanyang Technological University School of Biological Sciences Biosafety Level 3 (BSL-3) laboratory is set up as a core facility for research work involving biological materials of potential biohazard. Many human pathogens—viruses, fungi, parasites, rickettsia, and bacteria are classified as Risk Group 3 microorganisms in the Singapore national guidelines that require a BSL-3 facility for their storage and handling. A comprehensive approach to biosafety is based on a combination of administrative controls, safety practices and procedures, engineering controls, and personal protective equipment. For the benefit of other research institutes and future users in Singapore, here we report on facility requirements and implementation, management of biosafety and biosecurity measures, application of processes and procedures, and documentation specifically adopted in our facility.*

## Introduction

The primary purpose of biohazard containment is to minimize or eliminate exposures of laboratory personnel and the outside environment to potentially hazardous agents. Essential elements of Biosafety Level 3 (BSL-3) containment include standard microbiological practices, specialized safety practices which must be in keeping with the hazards associated with the agents and procedures, primary barriers consisting of safety equipment and containment devices, and secondary barriers such as laboratory facilities that are designed to protect personnel inside and outside the facility (U.S. Department of Health and Human Services et al., 1999; WHO, 2003). The backbone of the practice of biosafety is risk assessment. Infectious agents are classified into certain risk groups based on a number of variables (Health Canada, 1996; Johnson, 2000; U.S. Department of Health and Human Services et al., 1999; WHO, 2003). They include:

- Agent pathogenicity and infectious dose
- Consideration of exposure outcome
- Routes of infection

- Agent environmental stability
- Presence of a suitable host (human or animal)
- Information available from animal studies and reports of laboratory-acquired infections or clinical reports
- Any genetic manipulation of the organism which may alter infectivity and virulence
- Local availability of effective prophylaxis or therapeutic interventions

The Ministry of Health, Republic of Singapore has adopted guidelines regarding human pathogens, and these guidelines are used to determine the level of biosafety required for individual purposes. The guidelines include regulations regarding the import, transport, transfer, handling, and disposal of human pathogens and their risk group classification (Disease Control Branch, 2004). In Singapore, the BSL-3 facility is designed and designated for work with Risk Group 3 microorganisms and with large volumes or high concentrations of Risk Group 2 microorganisms that pose an increased risk of aerosol spread (Disease Control Branch, 2004; WHO, 2003). Risk Group 3, currently the highest risk level handled in Singapore, contains a growing list of human pathogens (Table 1). The use of BSL-3 facilities is not limited to research and development, but is necessary for all aspects of laboratory work including clinical, diagnostic, teaching, and production facilities in which agents are stored and handled that may cause serious or potentially lethal diseases as a result of aerosol exposure.

The Nanyang Technological University School of Biological Sciences Biosafety Level 3 facility (NTU-SBS BSL-3) is specifically designed for research involving biological materials of potential hazard. This facility has been designed to accommodate multipurpose-users with regular staff training and turnover; thus, user training plays a critical role. The adopted comprehensive approach to biosafety is based on a combination of administrative controls, safety practices and procedures, engineering controls, and personal protective equipment. Measures used to ensure security of the facility will not be discussed here due to their confidential nature. In the present climate where threats of bioterrorism and biological attacks

**Table 1**

Examples of Human Pathogens Classified in Singapore as Risk Group 3

Type	Microorganism
Viral agents	Hepatitis C virus Human immunodeficiency virus Japanese B encephalitis virus Rabies virus West Nile virus
Fungal agents	<i>Blastomyces dermatitidis</i> <i>Coccidioides immitis</i> <i>Histoplasma capsulatum</i> <i>Penicillium marneffeii</i>
Parasitic agents	<i>Echinococcus granulosus</i> <i>Leishmania brasiliensis</i> <i>Plasmodium falciparum</i> <i>Taenia solium</i>
Bacterial and Rickettsial agents	<i>Bacillus anthracis</i> <i>Brucella</i> —all species <i>Mycobacterium tuberculosis</i> <i>Rickettsia</i> —all species <i>Salmonella typhi</i> <i>Shigella dysenteriae</i> (Type 1)

are of global concern, the measures used to secure a facility storing high-risk agents is of utmost importance.

### Administrative Controls

All policy matters, operating procedures, and approval of research programs that involve the utilization of the NTU-SBS BSL-3 facility are the responsibility of the five-member Biosafety Committee headed by the Facility Director and assisted by the Facility Manager. The Dean of the School of Biological Sciences is the Facility Director. To ensure the smooth running of the facility, all users must first understand the administrative system and share duties and responsibilities in the facility. Prior to commencement of any research program within the NTU-SBS BSL-3 facility, approval from the committee is necessary. Principal investigators (PIs) are directly responsible for ensuring that day-to-day research work is conducted in a manner which minimizes the risk in the NTU-SBS BSL-3 facility. Principal Investigators are also required to ensure that:

- Performance complies with all standards of safety in the laboratory.
- Appropriate instruction and training of laboratory users has been completed (in collaboration with the Facility Manager).
- Appropriate immunizations and serological and/or

medical monitoring are administered to at-risk personnel

- Appropriate procedures for dealing with accident and emergency matters are in place (in collaboration with the Facility Manager).
- The committee is notified of modifications in program.

The Facility Manager directly assists the Facility Director and supervises the day-to-day operations of the laboratory. This includes dealing with accidental spills and emergency matters, maintaining consumables, general maintenance, and also annual decontamination of the laboratory. The manager is also responsible for maintenance and upkeep of the containment systems, support equipment, and waste disposal, and ensuring all operations are in accordance with the safety guidelines. All systems are regularly monitored with a checklist and documented. Administrative power is given to the Facility Manager to close the NTU-SBS BSL-3 laboratory in the event of any unsafe condition until the problem is corrected.

An annual inspection and audit, and preventive maintenance of the NTU-SBS BSL-3 laboratory is carried out to ensure all aspects of the integrity and safety features of the containment facility. External audit and inspection are exercised by the Singapore Ministry of Health. The NTU-SBS BSL-3 facility was tested and certified by a reputable consulting firm and certification is to

be renewed annually ensuring that the laboratory meets and, in many instances, exceeds the CDC/NIH (USA) guidelines (U.S. Department of Health and Human Services et al., 1999) for BSL-3 laboratory facility and WHO requirements (WHO, 2003).

### **Biosafety and Operations Manual**

A comprehensive Biosafety and Operations Manual was developed with input and direction from the Facility Manager to give full and precise details of the operational and safety procedures for the NTU-SBS BSL-3 facility. The procedures specified in the manual are standard operating procedures and must not be altered or substituted without proper evaluation and written approval by the NTU-SBS BSL-3 Biosafety Committee. The protocols and procedures within the manual are based on the CDC/NIH (USA) Biosafety Level 3 Standard Microbiological Practices and Special Containment Practices (U.S. Department of Health and Human Services et al., 1999). A requirement for all facility users is to understand all relevant protocols and procedures within the manual. All personnel entering the active facility for the purpose of storing and handling hazardous agents must fully read and comprehend the manual's contents.

### **Application Procedures**

The facility is designed to handle one agent at a time. Agent cross-contamination is inhibited by enforcing facility decontamination before a different agent is handled. Research groups are permitted to handle a single approved agent for an approved period only.

All research programs that require the use of the NTU-SBS BSL-3 facility are required to submit an application to the Biosafety Committee for approval before work can commence. The PI is responsible for completion and submission of the following forms, all of which are contained in the Biosafety and Operations Manual:

- “Notice of Intent to Work with a Hazardous Biological Agent or Toxic Material” (completed by the PI) requires information on all personnel associated with research projects, hazardous and infectious agents details, and protocol descriptions.
- “Authorization for Entry into NTU-SBS BSL3 Laboratory” (completed by all intended users) requires that the following have been performed: training and orientation to the facility, understanding of biosafety procedures and emergency matters, and reading and understanding of the Biosafety and Operations Manual.

The Biosafety Committee reviews and approves individual applications on the basis of scientific merit, risk assessment, and biosafety standards. Research projects involving high-risk organisms are then forwarded to the Singapore Ministry of Health for approval. Additionally, approval from the Agri-Food and Veterinary Authority of Singapore is required for handling of zoonotic agents.

Personnel intending to work within the NTU-SBS BSL-3 facility must have prior experience or training in standard microbiological practices and techniques. Prior to working in the laboratory, the user is required to undergo training and orientation on standard operating procedures conducted by the Facility Manager. Training particulars must be well documented. An approved user-record containing particulars of personnel who have been allowed to utilize the NTU-SBS BSL-3 laboratory must be properly maintained.

### **BSL-3 Laboratory Access**

The typical two-person rule applies whereby no individual is permitted to work alone in the laboratory. BSL-3 laboratory access requires the approval of the Biosafety Committee and administrative power is given to the Facility Manager for supervised admittance of all individuals involved in maintenance and equipment repair. At the laboratory access points, the international biohazard warning symbol and sign are displayed (Figure 1). Several security-pass systems are in place. Authorized users are fully briefed on all security systems implemented to control entry into the facility. No animals and plants are permitted to enter the laboratory. All materials and equipment taken into the laboratory must be decontaminated or sterilized before removal. No unauthorized or untrained personnel are permitted to enter the laboratory at any time. Written permission for guests to enter the laboratory must be obtained from the Facility Director or the Facility Manager. Guests are informed of the particularly hazardous nature of work and accompanied by authorized personnel. No person under 16 years of age is permitted in the laboratory. Authorized user's name, date, and time of each entry and exit are registered.

### **Safety Practices and Procedures**

#### **Personal Protection**

All personnel entering the laboratory must be in protective clothing. Personal protective equipment (PPE), including a solid-front disposable gown, shoe covers, hair cover, and gloves, must be worn at all times to work in the laboratory. Respirator and goggles are applied whenever required. Eating, drinking, smoking, storing food and drinks in the laboratory, handling contact lenses, and applying cosmetics are prohibited. Personal safety is further increased by washing hands properly and frequently and showering before leaving the laboratory, if required.

Each person must undergo an appropriate preplacement medical evaluation before commencing work in the facility. A baseline serum sample is obtained and stored for future reference. Illness which results in overt or potential exposures to infectious materials is immediately reported to the PI, the Director, and the Facility Manager. A document for illness report is adopted. This docu-

### Figures 1a and 1b

The international biohazard warning symbol and sign are clearly displayed at the doors of the laboratory.



(a)



(b)

ment provides the possible cause of infection, duration of exposure to infectious agents, potential contacts, and medical evaluation and recommendation.

#### Essential Biosafety Equipment

In accordance with standard practices, plastic disposable laboratory-ware is used whenever possible. Pipetting by mouth is prohibited and pipetting aids are provided. To minimize aerosol contamination, all handling of infectious materials is performed inside a Class-II Type B1 biological safety cabinet located inside the laboratory and maintained accordingly (U.S. Department of Health and Human Services et al., 2000). All procedures must be performed carefully to minimize the creation of aerosols. Centrifuge buckets are equipped with safety caps, and loading and unloading of infectious materials are performed in the biological safety cabinet. A high degree of caution is always taken with contaminated sharp items. Sharps are kept to the minimum in the laboratory and disposed of in puncture-proof sharps disposal containers. Eye wash stations, hand-wash basins, shower, biological spill kit, chemical spill kit, and fire blanket and distinguisher are available in the facility.

#### Storage of Infectious Agents

All infectious agents are stored securely in locked freezers within the laboratory, and storage areas are marked with the universal biohazard symbol. Storage containers are capable of withstanding the thermal shock of freezing and thawing. Each container is labeled with the identity of the infectious agent, the date of preparation, the name of the responsible person, and a reference number. Transfer and transportation of infectious agents are

made in a second container that is unbreakable with a leak-proof cover. An inventory of agents kept in the laboratory is maintained. Careful and impeccable documentation is important for biosecurity to prevent improper release of infectious agents from the laboratory.

#### Decontamination and Waste Management

Decontamination and waste management are handled according to WHO guidelines (WHO, 2003), enforced by the Singapore Ministry of Health. The following disinfectants are used in the laboratory. The range of materials reflects the variety of agents stored and handled in the facility:

- *Sodium hypochlorite*: 1 % solution for discarded jars, spillages, and disinfection of nonmetal utensils
- *Sodium hypochlorite*: 0.1 % solution for mopping the floor
- *Potassium Peroxymonosulphate (Virkon, Dupont)*: 1 % solution for bench top, biological safety cabinet, centrifuge buckets, and adaptors
- *Alcohol*: 70 % for general decontamination

A 110-litre autoclave is provided in the laboratory to sterilize all contaminated materials before leaving the laboratory. Disposable materials and infectious solid wastes are autoclaved then collected by licensed contractors as general nonhazardous wastes. Liquid waste (nonradioactive) is decontaminated with 1% sodium hypochlorite then autoclaved before disposal in the sink. Radioactive waste is stored until radiation levels reach background and then treated as normal biohazardous waste. Gaseous decontamination of the laboratory is required before any maintenance of scientific or communication equipment.

## Emergency Procedures

The project PI or Facility Manager is informed immediately if an accident or biohazardous spill involving agents handled in the BSL-3 facility occurs. All relevant contact numbers are posted in the laboratory. Emergency procedures are managed under the supervision of the PI or Facility Manager. Trainee facility users are thoroughly instructed on all relevant procedures for managing such biohazard spills during training and orientation to the BSL-3 facility. A detailed record of accidents and relevant clean-up information is held by the Facility Manager.

## Pest Control

Under the guidance of the Singapore Ministry of Health and CPG Consultant Private Limited, an integrated pest management (IPM) program has been implemented. The program minimizes reliance on the use of pesticides and emphasizes management of the environment. The rigorous sanitation and maintenance requirements associated with operating the facility preclude the establishment of pest “infestation” within the containment area. The primary pest control tactics used are nonchemical (i.e., traps, exclusion, physical removal, or disposal). This eliminates the potential for drift and volatilization of petroleum distillate and solvents associated with the use of some solid and aerosol formation pesticides. Pest management surveys inside the containment area will be conducted upon request by the Facility Manager in order to diagnose and resolve specific pest issues. An IPM logbook is compiled to indicate all pest management products that have been used in the facility.

## Engineering Controls

The NTU-SBS BSL-3 laboratory was built into the second storey of the Animal Facility of Nanyang Technological University School of Biological Sciences. This building is self-contained and free-standing. The electrical and laboratory equipment is installed for operating the facility as a BSL-3 laboratory, complying with the guidelines of the USA CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* (U.S. Department of Health and Human Services et al., 1999). The power supply is secured by a backup system.

## Building Design

The NTU-SBS BSL-3 facility consists of three compartments separated by doors that are linked with an interlocking system so that two doors cannot be opened simultaneously. The three required compartments are the Airlock Room, the Ante-Room, and the laboratory.

1. A positively pressurized Airlock Room ensures that no external humid air is drawn into the laboratory and resulting in condensation. Carbon dioxide cylinders necessary for incubators are located in the Airlock Room (Figure 2).
2. The Ante-Room, located between the laboratory and Airlock Room, is negatively pressurized with reference to both the Airlock Room and outside. A small shower room is attached.
3. The laboratory is negatively pressurized with controlled air flow and equipped with a hard-ducted biological safety cabinet which serves as the primary containment device for work with Risk Group 3 agents.

## Figure 2

Carbon dioxide cylinders are located in the Airlock Room and connected to incubators inside the laboratory. This enables cylinders to be exchanged without entering into the laboratory.



The walls of the laboratory are constructed of calcium silicate boards. The ceiling is made of steel plates with honeycomb material in between and supports human weight to allow access to the ceiling. The electrical and control devices are located above the ceiling allowing maintenance services to be carried out with minimum disruption inside the laboratory (Figure 3). The floor is layered with a 6-mm self-leveling chemical-resistant epoxy screed with coved corners. The walls and ceiling are coated with epoxy. All penetrations for services and ducts into the laboratory are sealed for air-tightness. The benches are made of chemical-resistant phenolic tops.

Communication devices such as telephone, fax machine, and computer with Internet access are available in the laboratory. A closed-circuit television surveillance system with two infra-red cameras inside and two cameras outside the laboratory is installed.

### System Operation

Negative pressure within the facility is achieved by supplying less air into the room than the volume of air exhausted via the biological safety cabinet and room general exhausts. Positive pressure is achieved by supplying more air into the room than the volume of air exhausted. A dedicated control system continuously monitors and maintains the preset flow rate difference between the supply and exhaust air to achieve the required room pressurization. The supply air is filtered at the Air Handling Unit to extend the life-span of the exhaust high-efficiency particulate air (HEPA) filters. All air exhausted from the laboratory is filtered through HEPA filters (Figure 4).

### Systems Checklist

Environmental conditions within the facility are closely monitored and recorded using systems checklists. The required conditions are checked and recorded regularly. Audible and visual alarms are triggered when room pressures are incorrect (Figure 5). The operating conditions necessary for each type of room within the facility are:

<i>Air Lock Room</i>	
Temperature	22+/-1°C
Relative Humidity	55+/-5%
Pressure	+7.5 pa
Air-change per hour	15
<i>Ante-Room</i>	
Temperature	22+/- 1C
Relative Humidity	55+/-5%
Pressure	-15+/-2 pa
Air-change per hour	15
<i>BSL-3 laboratory</i>	
Temperature	22+/-1°C
Relative Humidity	55+/-5%
Pressure	-20+/-2 pa
Air-change per hour	15

### Discussion

With Singapore's emphasis on research and development in life sciences and the regional and global spread of emerging infectious diseases, BSL-3 facilities with an efficient and comprehensive biosafety system are critical in keeping Singapore, and indeed the rest of the world, free from dangerous diseases. The BSL-3 practices em-

**Figures 3a and 3b**

Walk-on interstitial space above the ceiling of the laboratory. Maintenance and services can be performed without entering the BSL-3 laboratory.



(a)



(b)

**Figures 4a, 4b, and 4c**

(a) and (b) Both supply air and exhaust air are HEPA filtered.  
(b) Exhaust air from the laboratory and biological safety cabinet are not recirculated but discharged through HEPA filters to outside the building.



(a)



(b)



(c)

**Figures 5a and 5b**

A ventilation system establishes a directional air flow from access spaces into the laboratory.

(a) The laboratory and anteroom are negatively pressurized and room conditions are monitored by a checklist every day.  
(b) Audible and visual alarms are triggered when room pressures are incorrect.



(a)



(b)

ployed by NTU-SBS are designed to meet national and international standards. All aspects of the facility were carefully planned and inspected before certification and compliance were issued by an internationally recognized organization. The certification is accredited by the Singapore Ministry of Health. Protocols and procedures for the handling and possession of human pathogens in the facility comply with the guidelines established by the ministry. Furthermore, regular inspection and auditing are also performed by the ministry to ensure the safe and efficient operation of the facility. In the case of an infectious microorganism falling within the list of pathogens controlled by the Agri-Food and Veterinary Authority (AVA), approval from AVA must be sought before the pathogen may be stored or handled.

One of the most important factors that must be considered when planning laboratory work with potentially infectious agents is the risk-group classification. Even though there are global standard laboratory practices and many aspects of laboratory culture are shared throughout the world, the risk-group classification of hazardous agents does vary from country to country. Therefore, it is imperative that the risk group classification of a particular agent of interest is determined for the country of use. It is not uncommon, for example, that an agent classified into Risk Group 2 in one country may be classified as Risk Group 3 in another. For example, Dengue Virus Type 1-4 is classified as Risk Group 2 in Singapore, Canada, and Australia but Risk Group 3 in Belgium and the European Union (ABSA, 1988; Disease Control Branch, 2004).

Infections can be contracted in a variety of ways—by pricking the skin, splashing the eyes, contaminating the mouth, or inhaling fine aerosol droplets containing infectious particles. Aerosols not visible to the naked eye are produced by almost any procedure involving liquids, such as shaking, homogenizing, sonicating, centrifuging, pouring, and pipetting. Human error, poor laboratory technique, and misuse of equipment cause the majority of laboratory accidents, injuries, and work-related infections. Understanding and appropriate use of laboratory equipment, wearing suitable personal protective equipment, and standard microbiological procedures and practices are important safety measures all laboratory workers must apply. Complete safety requires all laboratory users to observe correct procedures and practices and to accept their responsibility to protect themselves and their fellow workers and environment. Another important factor for ensuring personal safety within a BSL-3 facility is that users maintain a calm yet cautious approach. The experiments should not be carried out either in a rushed manner or by a mentally or physically tired worker. The risk of personal injury and environmental accident is greatly reduced when workers have planned adequate time for experimental work and associated cleaning protocols. Finally, users must be honest and considerate of others.

Since it is difficult to detect one's misdeed that may create serious consequences, honest and prompt reporting of any accident must be part of the working culture in a BSL-3 facility.

In addition to the biosafety measures surrounding the BSL-3 facility which are the responsibility of the users and Biosafety Committee, the one other issue that the entire community must be vigilant towards is biosecurity. Biosecurity refers to measures taken to ensure the protection of biological materials, particularly hazardous agents and culture collections, from theft, illicit use, or intentional release. Restricted access to the facility, appropriate secure storage of agents, and strict inventory control of organisms aid in creating a secure facility. A closed-circuit television surveillance system has also been adopted by NTU-SBS. Currently, no device detects pathogens being removed from a facility, and the relatively small size of vials containing potentially lethal doses of agents results in a situation whereby present security measures are not optimal and can be improved. However, effectiveness of current biosecurity measures depends on the integrity of the individuals with access to the pathogens and, therefore, relies on collaborative effort between scientists.

## **Conclusion**

The design, building, and certification of a BSL-3 facility in Singapore is an expensive exercise in terms of finance, time, and organization. It demands a continuous effort and many steps to achieve the ultimate goal; however, the governing bodies and collaborating partners have led to the NTU-SBS BSL-3 facility becoming a functional, stand-alone unit. A key aspect of the efficient running of such a multiuse unit is the documentation and records used to monitor personnel, environmental conditions, maintenance, and so on. A minute failure in numerous operating systems can cause significant problems such that it may be deemed necessary to shut down the facility. Of equal concern is that the reputation of the institute or country and our professional integrity will be affected. Therefore, biosafety and biosecurity measures, no matter how small or how big, must be taken seriously by all involved.

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## Fact Sheets on Terrorist Attacks

The U.S. National Academies of Science has prepared fact sheets to provide reporters with reliable information on biological, chemical, nuclear, and radiological attacks. This effort was a collaboration with the U.S. Department of Homeland Security, and the Radio and Television News Directors Foundation. ABSA members may find the information useful in educational efforts on emergency planning.

The fact sheets can be found at [www.nae.edu/factsheets](http://www.nae.edu/factsheets).

**Biological Attack** (pdf file, 277 KB)—Where do biological agents originate? What’s the difference between “infectious” and “contagious”? How long after exposure will symptoms appear?

**Chemical Attack** (pdf file, 72 KB)—What are the different origins of toxic chemicals that could be used? How do chemical toxicities vary? What are the practical steps to take if there’s a chemical release?

**Radiological Attack** (pdf file, 68 KB)—What are radiological dispersal devices, a.k.a. “dirty bombs”? How are they different from nuclear bombs? What are their physical and psychological health effects?

**Nuclear Attack** (pdf file, 192 KB) **NEW!**—What is radioactive fallout, and how is it dangerous? What are the short-term and long-term effects of radiation exposure? What is the likely size of a nuclear explosion from an attack by terrorists?