In-house BSL-3 User Training: Development and Implementation of Programme at the Nanyang Technological University in Singapore

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Introduction

All potential users are required to have appropriate training before they start working inside the Nanyang Technological University School of Biological Sciences (NTU-SBS) BSL-3 laboratory. This policy statement is clearly defined in the Biosafety and Operations Manual. An in-house training programme has been developed and implemented in accordance with the training policy drawn up by the Institutional Biosafety Committee (IBC). The programme is expected to equip users with sound foundations for working safely in the BSL-3 laboratory. Continuing efforts to upgrade knowledge or skill in biosafety measures and nurturing appropriate safety practices are ultimate goals of the training programme.

Users must strictly comply with standard operating procedures (SOP) implemented at the facility. Related legislation and regulations play crucial roles and users need to know them. Additionally, international guidelines and circulations or instructions issued by local authorities also apply. No two BSL-3 facilities are the same in design and structure, administrative processes, and adopted biosafety practices. Orientation for a particular facility will be unique and a user’s familiarization with the facility will be of great value. Any training program implemented should meet the requirements indicated above. This article reports the impacts and contents of the in-house training program for the benefit of future users and newly developed facilities.

Training Requirements

The World Health Organization (WHO) mentions in the Laboratory Biosafety Manual, 3rd edition (WHO, 2004), that a continuous on-the-job safety training programme is essential to maintain safety awareness among laboratory and support staff. Training objectives are observable behaviors that the trainee is expected to demonstrate after training and constitute the conditions under which certain activities or behaviors are performed at the required level of proficiency. Training provides fundamental knowledge or skill that the trainee must master to be able to meet the behavioral objectives. Effective training must take into account the characteristics or attributes of the trainees. Individuals and groups may differ in aptitude, literacy, culture, and pre-training skill levels.

Training for BSL-3 staff is mandated under the Biological Agents and Toxins Act (BATA), which has been in force in Singapore since 2006 (Singapore Statutes Online, 2006; Tun et al., 2007). The Ministry of Health (MOH) also releases circulars or instructions regarding BSL-3 issues whenever required. Every facility appoints a biosafety coordinator who participates in the structured biosafety training course conducted by a MOH-approved training provider (ATP) and who must pass the competency test administered by the MOH. The biosafety coordinator acquires experience in the area of biosafety management and undergoes such training as the Director of Medical Services requires from time-to-time. He or she must be a member of the institutional biosafety committee that formulates safety measures, programmes, codes of practice, and all policies including training of staff. Training courses for personnel who work in a BSL-3 facility can be conducted by in-house personnel or by experienced external trainers if the institute does not have expertise in training.

NTU-SBS BSL-3 Facility

The NTU-SBS BSL-3 facility is specifically designed for research projects involving potentially hazardous biological materials (Tun et al., 2006). Appropriate staff training plays a critical role, since the facility accommodates multi-purpose users. All policy matters, operating procedures, review of risk assessment and approval of research proposals, and biosafety training and approval of authorized users are major responsibilities of a six-member Institutional Biosafety Committee headed by the Facility Director and assisted by the Facility Manager (FM). The Chair of the School of Biological Sciences is the Facility Director. A comprehensive Biosafety and Operations Manual has been developed to give full and precise details of the operation and safety procedures. The Facility Manager, who is also the MOH-approved Biosafety Coordinator, implements safety policies, relevant programs, and codes of practice formulated by the IBC, and organizes the in-house BSL-3 training course.

In-house Training Program

The in-house training program has been approved by the IBC to train potential users of the NTU-SBS BSL-3 laboratory. Program procedures consist of lectures, prac-
tical demonstrations and simulations, video learning, discussions, reviews and evaluation of training, and documentation. Training contents address related legislation, regulations, guidelines and facility requirements; biosafety culture and concepts; standard procedures and laboratory practices; laboratory instruments and their maintenance; emergency responses; administrative requirements; and application procedures or protocols adopted at the facility.

Who are authorized users?

Authorized users at the NTU-SBS BSL-3 facility are members of research groups headed by principal investigators (PI), including research scientists and postgraduate students. Projects by undergraduate students usually do not involve high-risk biological agents and they are not allowed to use the facility. The principal investigators and research group members are encouraged to attend the biosafety course organized by the Asia-Pacific Biosafety Association (A-PBA). It is the only MOH-accredited biosafety course locally available and is taught once a year. The A-PBA creates a strong trainer team by inviting many biosafety experts and experienced biosafety engineers from local and overseas institutions or associations such as the American Biological Safety Association (ABSA) and the Canadian Science Centre for Human and Animal Health. Many participants from Singapore and Southeast Asian countries take part in the intensive training which usually takes about 1 week followed by an examination at the end of the course. However, researchers who have attended such biosafety trainings and acquired basic biosafety knowledge still need to complete the in-house training course successfully. Then, they are registered as authorized users who have been approved by the IBC to work in the BSL-3 laboratory. The training sessions are conducted for two or three trainees at a time so that individual attention can be achieved.

Individual learning preferences are very important to achieve effective training. Trainees may differ in aptitude, literacy, culture, language, and skill and/or knowledge levels. Some are more visual or hands-on learners; others learn well from written materials. Some learn fast; some are slow learners. Any special needs of trainees must be addressed, such as course adaptation for those with hearing impairments. Using small trainee groups tends to reduce difficulties due to individual differences and maximize the effectiveness of the biosafety training. How the training programme is viewed by trainees in terms of improving their performance or personal safety may dictate the approach used.

Legislation, Regulations, and Guidelines

A good regulatory framework strengthens biosafety and biosecurity. Biosafety rules are strict and severe penalties are defined for offenders convicted of the unauthorized use of biological agents and toxins. All individuals who work in a BSL-3 environment must understand relevant legislation. Training elements must include complete and precise information about existing rules. It is also important to cover international guidelines and instructions occasionally issued by local authorities or government agencies.

The BATA clearly defines facility requirements for handling biological agents, provides for a comprehensive system of control, minimizes acts of bioterrorism by controlling the use of high-risk hazardous agents, and establishes a strong national biosafety culture. Major components of the BATA include lists of regulated biological agents and toxins; controls of importation, possession, transshipment and transfer; and transport requirements. The BATA adopts a schedule system for risk group classification of biological agents.

On recommendation of the National Biosafety Committee (NBC), the MOH has adopted the Laboratory Biosafety Manual (WHO, 2004) as the national guideline for biosafety to supplement the BATA. The manual provides expert guidance for developing specific codes of practices for the safe handling of potentially hazardous agents.

To work with zoonotic agents, approval for possession of biological agents from the Agri-Food Veterinary Authority (AVA) of Singapore is required. The AVA and the MOH have streamlined the import permit processing on the Singapore Custom Tradenet System. An importer is required to declare a set of MOH-AVA product codes for each zoonotic agent (AVA, 2005).

For genetically modified organisms (GMO), approval must be sought from the Genetic Modification Advisory Committee (GMAC) before submitting an application for approval and permit to the MOH (GMAC, 2005). Packaging the biological agent for transport requires the basic triple packaging system comprised of a primary receptacle, secondary packaging, and outer packaging. Biological agents are packaged to meet the International Air Transport Associations (IATA) regulations.

Following are some examples of international and local regulations the trainees need to know well.

Facility Requirements

The design, building, and certification of a BSL-3 facility are expensive exercises in terms of finances, time, and organization. Users should conceptually understand the system operation and purposes of the facility's structure and design. Knowing how things work will help trainees appreciate the facility requirements.

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 first room is a positively pressurized Airlock Room with a main entrance door. The second room is a negatively pressurized Anti-Room located between the laboratory and the Airlock Room. The laboratory is negatively pressurized with controlled directional air flow. All air exhausted from the laboratory is filtered...
Table 1
Application procedures and sharing of responsibilities at the NTU-SBS BSL-3 facility.

<table>
<thead>
<tr>
<th>No.</th>
<th>Activity</th>
<th>Responsible</th>
</tr>
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</table>
| 1   | Proposal for use of BSL-3  
• Application forms  
• Biological Agent/Risk Assessments  
• Complete protocol  
• List of laboratory users | PI |
| 2   | Review for approval  
• Review the proposal  
• Review the Risk Assessments  
• Approve the proposal  
• Initial evaluation of PI's and staff's training needs | IBC |
| 3   | Approvals from MOH, AVA, etc.  
• Approval to possess and handle the BA  
• Approvals for transfer/import/transport  
• Other regulatory communications | FM |
| 4   | Determination of training needs  
• Evaluation of work-specific SOPs and instructions  
• Determination of exact needs and duration of training  
• Requirements of external training providers (ATP)  
• Approval by BC | IBC |
| 5   | In-house training for users  
• Biosafety procedures and practices  
• BATA requirements  
• Orientation to NTU-SBS BSL-3 facility  
• Reading, understanding, and practicing the Manual  
• Go through generic and specific SOPs  
• Final assessment must be signed. | FM |
| 6   | Medical surveillance  
• Pre-medical checkup  
• Baseline blood sample of users  
• Immunization/medical treatments | PI |
| 7   | Facility system controls  
• Containment system and Biosafety control  
• Supply equipment and PPE  
• Emergency matters – biological spill, accidents | FM |
| 8   | Operational controls  
• Compliance with SOPs, guidelines, regulations  
• Standard microbiological practices and special containment practices  
• Waste management and housekeeping  
• Emergency matters – biological spills, accidents | PI and Authorized Users |

Table 2
Medical Surveillance Program for BSL-3 Authorized Users
All NTU-SBS BSL-3 potential users must undergo a medical checkup before working inside the laboratory. A recent pre-employment medical checkup may be applied, but some more activities will still be required.

<table>
<thead>
<tr>
<th>Requirements</th>
<th>Purpose</th>
<th>Responsibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health check and physical examination</td>
<td>Users need medical fitness.</td>
<td>The user sees the Clinic Doctor (NTU Medical Centre) with a request endorsed by BSL-3 Manager. Staff can claim reimbursement.</td>
</tr>
</tbody>
</table>
| Vaccination/Immunization | Basic vaccines or specific to agent involved in project. | 1. To be done by Clinic Doctor.  
2. To provide information about agent by BSL-3 Manager/PI. |
| Baseline blood sample collection | Sample must be kept for future reference and available when required. | 1. Clinic will take blood and pass it to whom?  
2. Who keeps sample? – PI |
| Medical records | Must be confidential and available if required. | The clinic keeps all records and will provide proof in case of infection. |
| Medical contact card  
(Name, IC or photo, Contact person) | 1. User can inform that he or she works in BSL-3 laboratory.  
2. Doctor may contact for more information. | To be issued by SBS/OHR.  
(Contact person – BSL-3 Manager/Doctor NTU Medical Centre) |
| Incident report to MOH | To specify the laboratory-acquired infection involvement. | 1. User to follow procedures for suspected LAI.  
2. Doctor to monitor LAI and follow up.  
3. BSL-3 Manager to cooperate and report. |

Communication devices such as telephone, fax machine, intercom, and computer with Internet access are available inside the laboratory. Administrative requirements, for example laboratory access, audit, documentation, etc., and legal requirements, such as approvals or permits through high-efficiency particulate air (HEPA) filters before release to the ambient air. The following environmental conditions within the facility are closely monitored: pressure, temperature, and humidity. Audible and visual alarms are triggered when room conditions are incorrect.
from local authorities and the annual recertification process, are clearly discussed during training sessions.

**Laboratory Instruments and Maintenance**

Laboratory instruments should be used properly since they can be the source of laboratory accidents. Operating procedures for each laboratory instrument must be available for each user’s particular application. A successful training programme should ensure that trainees know how to effectively operate all laboratory equipment safely and perform maintenance when needed.

Users are instructed to handle all high-risk biological agents inside a class II type B-1 biological safety cabinet (BSC). Understanding the functions of a BSC, standard operating procedures, proper maintenance, and precautions will play a critical role for working safely. All experimental steps must be performed carefully to minimize the creation of aerosols. Centrifuge buckets are equipped with safety caps, and loading and unloading of the buckets with infectious materials must be performed inside the BSC. Proper use of mechanical devices for pipetting, shaking, mixing, and diluting must be emphasized during training. High-pressured equipment such as autoclave and gas cylinders demand special cautions and awareness to ensure safe operations.

**Emergency Responses**

In spite of safety programmes in a laboratory, accidents still happen. Biological spills tend to be the most common accident. Good safety programmes, training,

### Table 3

Authorized BSL-3 users are required to record when they enter the laboratory.

<table>
<thead>
<tr>
<th>BSL-3 LABORATORY</th>
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<tbody>
<tr>
<td>SCHOOL OF BIOLOGICAL SCIENCES</td>
</tr>
<tr>
<td>NANYANG TECHNOLOGICAL UNIVERSITY</td>
</tr>
</tbody>
</table>

**BSL-3/09: CRITICAL SYSTEMS CHECKLIST**

*(To record every day on use of laboratory.)*

**Outside the SBS BSL-3 Laboratory**

1. Blue indicator light in the lab is NOT on (Yes/No)
2. Pressure gauge reading (Airlock Room)

**Airlock Room**

3. Air-conditioning (On/Off)
4. Pressure gauge reading (Ante-Room)

**Ante-Room**

5. Air-conditioning (On/Off)
6. Pressure gauge reading (Laboratory)

**Laboratory**

7. Protective equipment available (Yes/No)
8. User’s log-book available (Yes/No)
9. Operations manuals available (Yes/No)
10. Shower room available (Yes/No)
11. First aid kit available (Yes/No)
12. Chemical spill kit available (Yes/No)
13. Biohazard spill kit available (Yes/No)
14. Alarm light (On/Off)
15. Air-conditioning (On/Off)
16. Biological Safety Cabinet functioning (Yes/No)
17. Autoclave functioning (Yes/No)
18. Telephone, fax, network, and intercom functioning (Yes/No)
19. Computer facility working properly (Yes/No)
20. Eyewash station available (Yes/No)
21. Fire extinguishers available (Yes/No)
22. Waste bins available (Yes/No)
23. Emergency contact numbers available (Yes/No)

Remarks on abnormal conditions observed:

<table>
<thead>
<tr>
<th>Signature</th>
<th>Name of Staff</th>
<th>Date</th>
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and serious precautions can help reduce the number, frequency, and severity of accidents. Emergency procedures should detail various preventive measures and operational actions to be taken when an emergency occurs at a BSL-3 facility. All possible scenarios including the worst cases that could happen in the laboratory should be identified and stressed in the training programme.

The NTU-SBS BSL-3 facility has developed an emergency response plan (ERP) comprised of standard operating procedures for various emergencies (Tun et al., 2008). The plan consists of many parts that provide useful information for all identified potential emergency situations. Required details include: description of the location and its neighboring areas; access and escape routes; the nature of applications involving biological agents, chemicals and reagents along with their hazard and risk assessments; descriptions of all possible emergency scenarios; required procedure execution, evacuation procedures, and notification of government agencies; important contacts and monitoring procedures; communication procedures; and support facilities and aid equipment for detection and protection. Trainees go through this manual in detail. Various types of emergency and laboratory accidents can be analyzed in video-viewing sessions. Exercises or simulated situations are conducted to provide hands-on experience for accidental procedures such as biological spill management, medical emergencies, and fire emergencies. Trainees get precise and correct information about what to do, whom to contact, what equipment is available for response, what steps to follow, and how safe evacuations take place during particular emergencies.

**Standard Procedures**

Human error, poor laboratory techniques, and misuse of equipment cause the majority of laboratory injuries and work-related infections. Standard procedures and good laboratory practices must be in place to prevent laboratory-acquired infections, to reduce laboratory accidents, and to promote biosafety and biosecurity measures.

The facility has adopted a *Biosafety and Operations Manual* with reference to the WHO manual for safety applications. Trainees go through the manual and all standard procedures in detail during training sessions. Procedures include learning how to perform proper waste management of solid, liquid, chemical, radioactive, or mixed wastes; how to handle sharp items; how to minimize aerosol generation during experimental steps; how to operate laboratory equipment; how to handle biological agents safely; and how to perform adequate housekeeping. Practical demonstrations and hands-on participations are used and are thought to be more effective when learning basic procedures such as gowning, de-gowning, and application of personal protective equipment; putting on

![Flow chart showing the steps in the application process for research on genetically modified organisms.](image-url)
and off gloves; proper use of the N95 mask and PAPR; adequate hand-washing, etc.

**Specific Protocols at the Facility**

Trainees undergo training for specific procedures or protocols in use at the facility because administrative controls and systems operations may differ from one facility to another. Laboratory access requirements, application procedures, and responsibility sharing (Table 1), medical surveillance programs (Table 2), guest or visitor policy, working hours, and documentation systems (Table 3) are some important examples.

Scientists and professional and academic personnel tend to shy away from documentation and paperwork. But BSL-3 administrative processes involve lots of documentation. Many factors on biosafety issues are considered for a research proposal involving high-risk agents. The IBC typically needs a vast amount of information for review, risk assessment, and the approval process. Application forms are complicated, and processes involve many steps and are time-consuming (Figures 1, 2, and 3). Such processes are prepared and put in place to meet the legal requirements and other administrative requirements. The PI and authorized BSL-3 users in the research group are essentially provided with information about specific procedures because their consideration and cooperation are critical. Laboratory documentation procedure details (for example what documents are in use and how to perform proper recording) are best explained by the ones who are actually doing the work.

**Training Schedules**

Contents of BSL-3 training are summarized in Table 4. Training hours, training procedures, and use of teaching aids will depend on the type of individuals and the
Importation of BA (All Schedules 1-5)

1. PI submits application to IBC
   - IBC application form
   - (Non-BSL-3 Users/BSL-3 Users)
   - Risk assessment
   - Detailed project protocol

2. Biosafety Coordinator
   - Apply the MOH for permit to possess
   - Coordinate with regulatory agencies (AVA/MOH)
   - May require assessment from GMAC
   - Submit documents to IBC for approval

3. IBC
   - Review and assess application
   - Approval (if required)

4. PI
   - Apply import permit through Tradenet
     www.tradenet.gov.sg
   - Furnish necessary documents
   - CR No. 74393060000G

Importation/Possession of GMO
MOH – Human health – under BATA
AVA – Under Animals and Birds Act

In-house training at the NTU-SBS BSL-3 facility has no regular or fixed schedule, so training is scheduled whenever the need arises. The facility manager arranges a training session when new users apply or when current users need retraining. Two or three trainees are in one session, so that individual attention can be achieved. About 25-30 hours, extending over a few days, are required to complete a training course. Prolonged training sessions without any breaks are avoided since the trainees may lose interest. The program consists of lectures, demonstrations, hands-on practical sessions, video viewing, and lots of discussions. Furthermore, many hours are needed for trainees to perform practical procedures such as application of PPE, waste management, spill management, housekeeping, and other hands-on operations. Such participation ensures that trainees have acquired good laboratory practices or habits, plus they can remind themselves of the possibility of human error and negligence. For maximum safety efforts, how they work is more important than what they know. However, performing correctly shows their understanding and awareness of biosafety. The facility manager spends a lot of time observing trainees’ behaviors and performance for evaluation purposes. He or she also enters the
laboratory together with new users to observe and assess how they work for the first 3–4 weeks of a new project. Users must show proper ways of working and satisfactory performance in various situations. On occasion, close mentoring has extended over a long period of time (months). Occasional checks are carried out to identify potential deficiencies in safety standards and documentation. These efforts help improve the development of a true biosafety culture and are a measure of the significant success of the training programme.

### Conclusion

Currently, one research group is using the facility for its project. Five authorized users, one principal investigator, two post-graduate researchers, and two PhD students have been trained. Actually, post-graduate researchers and PhD students work inside the laboratory. None of them had their access to the facility revoked due to failure to follow regulations and procedures. During the annual certification and shutdown period, users had the
opportunity to discuss biosafety issues with the AFC and to review their performance. During this period, all of the users were retrained before resuming their work. This was not due to failure to follow the established procedures, but to address the certifiers’ comments.

Biosafety and biosecurity are main concerns in BSL-3 environments. Knowing the procedures may not be good enough for BSL-3 users, because how they actually work is more important. The IBC takes such issues seriously and has developed a strict policy statement for laboratory users: Unapproved or disapproved procedures may not be carried out under any circumstance. Failure to observe this rule can result in removal of all access to the BSL-3 laboratory and may result in termination of employment or degree programmes. This kind of enforcement is necessary to ensure users have the proper awareness and attitude towards biosafety and biosecurity.

Common sense safety requires all BSL-3 users to observe correct procedures and practices and to accept responsibility to protect themselves, their fellow workers, and the environment. Safety must be the highest priority when working in a high-risk situation. One’s misdeed can affect others and generate bad consequences. Users should be honest, responsive, and considerate with others. Experiments should not be carried out either in a rushed manner or by a mentally or physically fatigued worker. The risk of potential personal injuries and environmental accidents will be greatly reduced when workers have planned adequate time for work and associated biosafety procedures. All important points are mentioned and discussed during training sessions. The Nanyang Technological University School of Biological Sciences (NTU-SBS) BSL-3 laboratory program operates on the premise that trainees should have a good attitude and habitually perform the required norms for biosafety. The in-house training is conducted to make trainees not only understand biosafety practices and procedures to follow, but also to accept biosafety culture as a way of life when conducting research with potentially hazardous biological substances.

References


Join a Committee

Have you ever considered joining a committee? When you choose to serve on a volunteer committee, you open a world of possibilities for networking, professional growth, and career opportunities while serving your profession. Volunteer member groups are the backbone of the association because they: serve as a forum for exchange of information; advance the science in all specialties of biosafety; develop guidelines and standards; provide education and training; and link ABSA to many other institutions.

You should explore committees in areas of the profession where you are active or have an interest. There is a great variety; you can be sure to find one of interest to you. Please review the list of committees and identify those areas in which you would like to participate or contact the chair of the committee (www.absa.org/abocommittees.html) that interests you to find out more information about the committee’s goals.