



Office of Safety, Health & Environment

NUS Laboratory Biorisk Management Manual

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DOCUMENT AMENDMENT AND REVIEW HISTORY

	DATE	REV. NO.	AMENDMENT / REVIEW	RECORDED BY
01	19 July 2013	4.0	Amendments: 1. Chapter 3 – inclusion of other relevant national regulations, namely: a. Animals and Birds Act (Section 3.3), b. Control of Vectors and Pesticides Act (Section 3.4), <i>and</i> c. Veterinary Biologics (Section 3.10). 2. Chapter 3 – update on: a. Training (Section 3.15), <i>and</i> b. NUS Occupational Health Programme (Section 3.16). 3. Chapter 4 – change of online portal for submission of Project Risk Assessments from Online Project Risk Assessment System (OPRAS) to integrated Online Research Compliance System (iORC), which was launched on 20 Nov 2012. 4. Chapter 5 – inclusion of other engineering controls, namely: a. laminar flow hoods, b. glove boxes, c. vacuum line chemical traps and filters, d. chemical fume hoods, <i>and</i> e. centrifuges. 5. Chapter 7 – inclusion of other good microbiological techniques & recommended work practices for common laboratory equipment, specifically: a. working with lentiviral vectors, b. safe handling of sharps, c. flow cytometers, d. blending, mixing, sonicating and cell disruption equipment, e. Bunsen burners, electrophoresis equipment, f. hot plates, drying ovens, and other heating devices, g. lyophilizers / freeze dryers, h. microscopes, i. microtomes / cryostats, j. pipettes and pipetting aids, k. refrigerators, freezers and cold rooms, l. ultraviolet (UV)-emitting equipment, <i>and</i> m. miscellaneous equipment (waterbaths and shakers). 6. Chapter 8 – additional information on: a. Selecting a Chemical Disinfectant, <i>and</i> b. Biological Waste Decontamination and Disposal. 7. New Chapters:	Dr. Lim Cheh Peng

CHAPTER 1 INTRODUCTION

1.1 OVERVIEW

Research and teaching activities in National University of Singapore (NUS) involves extensive use of various biological materials. Anyone working with biological materials in NUS must be aware of the potential risks associated with working with biological materials, and take the necessary precautions to prevent undue exposure to these agents, resulting in consequences such as laboratory-acquired infections.

The purpose of the NUS Laboratory Biorisk Management Manual is to detail the University's biological safety programme and to provide guidelines for all university personnel for the safe operation of laboratories involving biological materials. The policies, rules, and procedures set forth in this manual are developed with the purpose of promoting a safe environment for the protection of NUS employees, students, visitors, and community. The manual also provides guidance on the procedure for risk assessments in a biological setting and the appropriate controls to be adopted in the management of risks. Many of the practices and requirements mentioned are basic codes of practice essential for conducting research with hazardous or potentially hazardous biological materials and are also established with reference to the World Health Organization (WHO) [Laboratory Biosafety Manual, 3rd Edition](#). The codes of practice are also developed to align with recommendations and specific provisions stipulated by regulatory agencies in Singapore.

This manual should be used in conjunction with other laboratory safety manuals, i.e.:

- NUS General Laboratory Safety Manual – provides safety and health requirements on issues common to all laboratories, for example, commissioning and decommissioning of laboratory, laboratory sign posting, personal protective equipment, first aid, contractors management, etc.
- [NUS Fire Safety Manual](#) – provides fire safety procedures and requirements based on Singapore regulations, national and international codes.
- [NUS Laboratory Chemical Safety Manual](#) – provides safety and health requirements for working with chemicals in laboratories.
- [NUS Laboratory Ionizing Radiation Safety Manual](#) – provides safety and health requirements for working with ionizing apparatus and radioactive materials in laboratories.
- [NUS Laboratory Laser Safety Manual](#) – provides safety and health requirements for working with laser producing equipment in laboratories.
- [NUS Occupational Health and Safety Manual for Personnel with Research Animal Contact](#) – provides safety and health requirements pertaining to animal research.
- [NUS Safety and Health Manual for Field Trips](#) – provides guidance on risk assessment methodology / safety considerations for field trip and its related activities.
- [NUS Laboratory Ergonomics Manual](#) – provides guidelines to support a safe and healthy work environment by minimizing the risk of developing musculoskeletal disorders due to ergonomic hazards.

The guidelines in this manual should be read before work in the laboratory is initiated. It details good microbiological practices which include aseptic techniques and other biological laboratory safety practices that are necessary to prevent contamination of the laboratory with the agents being handled and contamination of the work with agents from the environment. It is essential that laboratory personnel are trained and proficient in the practices and techniques required for handling biohazardous

material. Eventually, all personnel working in the laboratories should know how to identify hazards, minimize risks and carry out their laboratory work safely.

1.2 EMERGENCY PHONE NUMBERS AND SAFETY PERSONNEL CONTACTS

1.2.1 Emergency Telephone Numbers

Ambulance/Fire	995
Police	999
Campus Security (24 hours)	x1616 (6874 1616)
General Maintenance/Breakdown of Services (24 hours)	x1515 (6516 1515)

1.2.2 University Health Centre (UHC)

University Health Centre (UHC)

20 Lower Kent Ridge Road
University Health Centre, Level 1
Singapore 119080
Tel: (65) 6776 1631
E-mail: uhc_health@nus.edu.sg
Website: <http://www.nus.edu.sg/uhc/>

Operating Hours

Monday – Thursday	8.30 am – 6.00 pm
Friday	8.30 am – 5.30 pm
Sat, Sun & Public Holidays	Closed

Closed for lunch from 12.30 pm - 1.30 pm.

Last registration is 30 minutes before closing time.

For medical examination that require lab test and X-ray, please register between 8.30 am - 11.30 am or 1.30 pm - 4.30 pm.

1.2.3 Nearest Hospital

In the event of critical injury / illness after office hours, proceed to the Accident & Emergency Unit of a nearby hospital. The nearest hospital in the vicinity of the University is:

National University Hospital (NUH)

Lower Kent Ridge Road
Singapore 119074
Main Line (24 hours general enquiries) Tel: (65) 6779 5555
Emergency Tel: (65) 6772 5000
Website: <http://www.nuh.com.sg>

1.2.4 Office of Safety, Health and Environment (OSHE)

- a. **Office of Safety, Health & Environment**
Ventus (University Campus Infrastructure)

8 Kent Ridge Drive
#03-02
Singapore 119246
General enquiries: 6516 1084
Fax: 6774 6979
E-mail: safety@nus.edu.sg
Website: <http://www.nus.edu.sg/osh/>

b. **Faculty/ Department Safety & Health Officers / Coordinators**

Contacts for Safety & Health Officers / Coordinators on safety and health issues pertaining to your faculty/department are assessable at [Office of Safety, Health & Environment](#).

1.2.5 Occupational Health Clinic (OH Clinic)

Occupational Health Clinic

Office of Safety, Health & Environment (OSHE)
University Health Centre, Basement
20 Lower Kent Ridge Road
Singapore 119080
General enquiries: 6516 7333 / 6601 1781

Operating Hours

Monday – Friday **8.30 am – 12.00 noon, 2.00 pm – 5.30 pm**

1.3 BIOHAZARDS AND POTENTIALLY INFECTIOUS MATERIALS

A biological hazard or biohazard is an organism, or substance derived from an organism, that poses a threat to (primarily) human health.

Categories of biohazards or potentially infectious materials include:

- a. Human, animal and plant pathogens
 - Viruses, including oncogenic and replication-defective viruses (including their naked viral DNA or RNA genome)
 - Bacteria, including those with drug resistance plasmids
 - Fungi
 - Parasites
 - Undefined or other infectious agents, such as prions
- b. All human blood, blood products, tissues and certain body fluids
- c. Cultured cells (all human or certain animal) and potentially infectious agents these may contain
- d. Allergens
- e. Toxins (bacterial, fungal, plant, etc.)
- f. Certain recombinant nucleic acid products
- g. Certain proteins (e.g. HIV TAT or HSV VP22 fusion proteins, etc.)
- h. Clinical and diagnostic specimens
- i. Infected animals and animal tissues

CHAPTER 2 BIOSAFETY PROGRAMME ADMINISTRATION

The Institutional Biosafety Committee (IBC) is the university level committee to oversee the development and implementation of the biosafety programme. The Office of Safety, Health and Environment (OSHE) is the administrator of this programme. The Biosafety Programme consists of the following elements:

2.1 ROLES AND RESPONSIBILITIES

2.1.1 NUS President

The President of the National University of Singapore represents the University as the Employer. The ultimate responsibility for safety and health policy and program rests with the President who may delegate the authority and the responsibility needed by the IBC, Deans and Heads of Departments to effectively supervise the occupational safety and health of staff under his or her management. The IBC and OSHE can report any incident or conditions of noncompliance to the President, Provost or Deputy President who are entitled to partially or fully close labs or facilities until all safety issues are addressed.

2.1.2 NUS Institutional Biosafety Committee (IBC)

The IBC is appointed by the President. The Terms of Reference for the IBC are:

- Review the SOPs, Standards and Guidance Documents at university, faculty and departmental level and recommend revisions to the Director of OSHE.
- Serve in an advisory capacity to OSHE on all Biosafety related matters.
- Establish procedures for the registration of biohazardous agents, and review the use of such agents and GMOs as required by the Genetic Modification Advisory Committee (GMAC).
- Approve all new projects involving biohazardous agents of Risk Group 2 and above through a risk assessment framework that must be completed by the respective Principal Investigators (PIs) before the commencement of a research project or teaching experiment.
- Review the NUS Biosafety Programme, as well as any audit and inspection findings conducted by OSHE or other independent parties on faculties and departments.
- Recommend to the NUS President on specific action items related to the Biosafety Programme.
- Perform the roles and responsibilities for Institutional Biosafety Committee stipulated in guidelines issued by the Genetic Modification Advisory Committee (GMAC).
- Advise the University management on BSL 3 and ABSL 3 policies and programmes.

2.1.3 Deans and Head of Departments (HOD)

All Deans and HODs of respective lab-based faculties and departments will ensure that their respective faculty or departmental biosafety SOPs, standards and guidance documents as well as components of the Biosafety Programme implemented at departmental and faculty level are in order and reviewed periodically. Faculty Safety Officers and personnel appointed to assume safety responsibilities shall be empowered by the respective Deans or HODs to coordinate the NUS Biosafety Programme at the faculty and departmental level.

2.1.4 Principal Investigator (PI)

Principal Investigators (PIs) / researchers are required to conduct risk assessment for activities involving biological materials. It is the responsibility of the respective PIs to ensure safe handling of such substances in his/her lab. The PI will be accountable for the inventory of biological agents in his/her laboratory.

PIs are required to be familiar with and follow national regulations and University level policies, directives and guidance documents. PIs who have in their possession agents designated under Risk Group 2 and above will be accountable for the inventory of such agents in his/her lab, and must register such agents with the IBC as part of their risk assessment submissions.

In performing the risk assessment using a common risk assessment framework established under the Biosafety Programme, PIs will document that protocols and facilities do not jeopardize the safety, health and well-being of themselves, their employees, students, collaborators, contractors and the general public. PIs should ensure that all personnel working in laboratories in which Risk Group 2 agents and above are handled are familiar with the relevant local and University level guidelines, and are appropriately trained and informed of the risks and hazards present in the lab under his/her charge. All risk assessment submissions are to be submitted to the respective Heads of Departments (HODs)/ Deans for endorsement prior to their submissions to the IBC.

The PIs are required to establish and maintain a Safety Management System for all research activities in his/her laboratory. It is also the PI's responsibility to provide the necessary resources and training needed to ensure good safety practices and adequate infrastructure for the safe operation of the lab.

2.1.5 Staff and Students

Under the Ministry of Manpower's Workplace Safety & Health Act, every person at a workplace is obligated to ensure the safety of their workplace and health of others in the workplace. All staff members and students must comply with all national regulations, and university level standards and guidance documents that are applicable to their area of work and ensure that they carry out their work safely.

Support staff such as maintenance service personnel (include NUS operations support staff and external contractors engaged for repair and/or maintenance of structure, facilities and equipment) as well as domestic cleaning service providers are also covered under the Act. Support staff should have the knowledge or be informed of the nature of work in the laboratory, and safety regulations and procedures of the University.

2.1.6 Office of Safety, Health & Environment (OSHE)

OSHE will provide administrative support to the IBC, maintain the NUS Laboratory Biorisk Management Manual, manage all registration and reporting processes for the IBC, maintain appropriate records, and serve as liaison with all faculties, departments and external agencies in the ongoing implementation of the University's Biosafety Programme.

OSHE will also coordinate the provision of biosafety training to relevant staff through the NUS Structured Safety Training System (SSTS). OSHE will arrange periodic biosafety audits and reviews on departments and faculties. OSHE will also be the University body tasked to coordinate any incident or accident investigations as called for by the IBC or the President.

2.1.7 University Health Centre (UHC)

University Health Centre (UHC) will provide primary healthcare, e.g. first aid cases and work-related incidents.

2.1.8 Occupational Health Clinic (OH Clinic)

The Office of Safety, Health and Environment (OSHE) is the general administrator of the Occupational Health programme by providing policies, standards and guidelines. The Occupational Health Division, under OSHE, will provide oversight on medical policies pertaining to biosafety work, conduct specialist OH Clinics to address the medical surveillance, treatment of work-related conditions, fitness to work requirements, and will also assist in the investigations and review of affected personnel in the event of lab incidents.

2.2 UNIVERSITY SAFETY AND HEALTH POLICY

The National University of Singapore (NUS) Safety and Health Policy is established to protect the safety and health of the University staff, students, collaborators, contractors and visitors.

Please refer to [General Safety and Health Policy](#) available at OSHE's website for more information.

2.3 UNIVERSITY BIOSAFETY DIRECTIVES

The NUS Safety Directive (Directive No. 0703) on *Staff and Students Working in BSL3 Facilities* defines the minimum requirements NUS staff or students need to comply with prior to working in BSL3 and ABSL3 facilities.

Please refer to [Directive No. 0703](#) for the full description.

2.4 SAFETY MANAGEMENT SYSTEM @ NUS DOCUMENT STRUCTURE

All NUS laboratories should align their procedures and practices to that of the University. Researchers should be familiar with all safe working procedures prescribed by OSHE, which are published in various OSHE manuals (see the list of OSHE manuals in Section 1.1). These are prescribed procedures and practices which are based on the local legislations and best practices in laboratories and should be adhered to at all times.

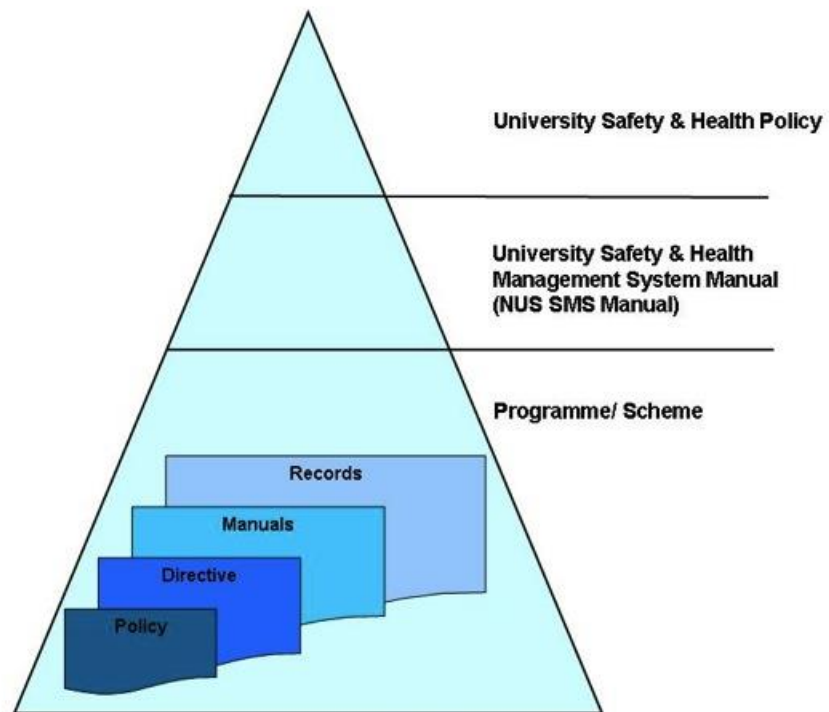


Figure 2.1 A schematic diagram on the document structure pertaining to Safety Management System @ NUS

CHAPTER 3 NATIONAL REGULATIONS AND NUS BIOSAFETY REQUIREMENTS

The following information describes the national regulations and guidelines, and the institutional / university-level biosafety requirements, for all researchers in the National University of Singapore undertaking laboratory-based research projects or teaching modules involving life sciences. It is the responsibility of each Principal Investigator to ensure that the laboratory is in compliance.

For Principal Investigators who are involved exclusively in field-research work, please refer to [NUS Safety & Health Manual for Field Trips](#).

NATIONAL REGULATIONS AND GUIDELINES

NUS Principal Investigators working with biological materials shall ensure that their work is in compliance with the national regulations described below and that the staff and students working under them are aware and comply with these requirements.

Please consult OSHE or Faculty Safety & Health Officers to assist in the application of relevant approvals, permits and licences.

3.1 WORKPLACE SAFETY AND HEALTH ACT (WSHA)

The Workplace Safety and Health Act (WSHA), administered by the Ministry of Manpower (MOM), came into effect on 1 March 2006 and it covers all workplaces. The Act stipulates the workplace safety and health obligations to be fulfilled, as well as responsibilities of every person in the workplace. The WSHA covers:

- The responsibilities of stakeholders (such as employers, principals¹, occupiers², employees)
- Hazardous substances
- Machinery and equipment

Under the WSHA, subsidiary legislations that are applicable to NUS include:

- [Workplace Safety and Health \(General Provisions\) Regulations 2006](#)
- [Workplace Safety and Health \(First-Aid\) Regulations 2006](#)
- [Workplace Safety and Health \(Incident Reporting\) Regulations 2006](#)
- [Workplace Safety and Health \(Risk Management\) Regulations 2006](#)
- [Workplace Safety and Health \(Confined Spaces\) Regulations 2009](#)
- [Workplace Safety and Health \(Medical Examinations\) Regulations 2011](#)

The regulations stipulate the following requirements in all workplaces:

- Conduct risk assessments to identify and control workplace safety and health issues
- Provide safe work facilities and arrangements for their workers

¹ Any person who engages another person or organization to supply labour or perform work under some arrangement other than a contract of service (definition extracted from A Guide to Workplace Safety and Health Act, Ministry of Manpower, Singapore).

² The person who has control of the premises regardless of whether he is the owner of those premises (definition extracted from A Guide to Workplace Safety and Health Act, Ministry of Manpower, Singapore).

- Ensure safety in the use of machinery, equipment, substances and in conducting work processes at the workplace
- Provide adequate instructions, information, training and supervision of workers
- Implement control measures for dealing with emergencies

With respect to infectious agents and biohazardous materials (substances which contain toxins, biological waste, spent culture medium, contaminated or infected blood, urine or faeces, tissues or organs, or animals), [Workplace Safety and Health \(General Provisions\) Regulations](#) stipulates the provisions of effective measures to protect the person from harmful effect for work involving exposure to any infectious or biohazardous material that may constitute a risk to the person's health. The Workplace Safety and Health (General Provisions) Regulations also stipulate the provisions for:

- Safe handling of hazardous substances,
- Warning labels,
- Safety data sheets,
- Safety precautions to prevent and minimize exposure to toxic dust and fumes,
- Ensuring personnel are not exposed to toxic substances in excess of the permissible exposure levels,
- Annual examination by authorized examiners of steam boilers such as autoclaves.

The [Workplace Safety and Health \(First-Aid\) Regulations](#) stipulate the need to provide sufficient number of first-aid boxes and the requirement of first-aiders in workplaces.

The [Workplace Safety and Health \(Incident Reporting\) Regulations](#) specify the conditions and duty to notify and report accidents leading to death or injury, dangerous occurrences or occupational diseases.

In NUS, all safety related incidents are to be reported to OSHE within 24 hours via [NUS Accident / Incident Reporting System \(AIRS\)](#).

Under the [Workplace Safety and Health \(Risk Management\) Regulations](#), every workplace is required to conduct risk assessments and establish reasonably practicable steps to eliminate, minimize or control the risks. Records of these risk assessments need to be maintained for a period of not less than 3 years. The risk assessments need to be reviewed at least once every 3 years or upon the occurrence of an accident in the workplace or when there is a significant change in work practices or procedures.

Under the [Workplace Safety and Health \(Medical Examinations\) Regulations 2011](#), workers who are exposed to the hazards listed in the regulation are required to undergo specific mandatory pre-placement and periodic medical examinations known as statutory medical examinations. All NUS staff and students who are exposed to these hazards should undergo medical examinations to be conducted by NUS Occupational Health (OH) Physician should they have significant exposure to those hazards.

Details on the Workplace Safety and Health Act and subsidiary legislations are available at the Ministry of Manpower's website on [Workplace Safety & Health](#). A summary of a [Register of Safety & Health Regulations Applicable to Laboratories](#) is also available at OSHE's website.

The key compliance requirement for Workplace Safety and Health Act is that all laboratories activities shall be subjected to risk assessments and that all PIs shall establish a safety management system for their laboratory operations.

3.2 BIOLOGICAL AGENTS AND TOXINS ACT (BATA)

The Regulatory Policy Branch (Biosafety Team) (previously known as the Biosafety Branch) at the Ministry of Health (MOH) was established in 2005 and is responsible for administering the Biological Agents and Toxins Act (BATA), which came into force on 3 January 2006 in Singapore. This Act intends to prohibit and otherwise regulates the possession, use, import, transshipment, transfer and transportation of biological agents, inactivated biological agents and toxins that are of public health concern.

Please refer to Singapore Statutes Online on [Biological Agents and Toxins Act](#) and [MOH's BioSafety website](#) for detailed information pertaining to the BATA.

3.2.1 Classification of BATA Schedules

Under the BATA, biological agents and toxins are classified into five schedules which define the requirements that are needed for possessing, handling, working and transporting the biological agent/toxin under each schedule.

First, Second, Third and Fourth Schedules include biological agents such as bacteria, virus, fungi and parasites capable of causing disease in humans while the Fifth Schedule includes a number of biological toxins that are of concern to human health. The classification of biological agents into different schedules correlates to the various risk levels associated with the agents and toxins. BATA biological agents of Risk Group 2 are grouped into Third and Fourth Schedule, whereas Risk Group 3 and Risk Group 4 are grouped under First and Second Schedule, respectively. All toxins regulated by BATA are grouped into Fifth Schedule.

Table 3.1 summarizes the classification of the five BATA Schedules and their corresponding biological agents and toxins.

Table 3.1 BATA Schedules with the corresponding description and number of Biological Agents and Toxins.

Schedule	Description	Number
First Schedule Part I	Risk Group 3 Biological Agents which can cause serious disease which is of high risk to the individual.	55
First Schedule Part II	Description is the same as First Schedule Part I but they also have the potential to be weaponised.	23
Second Schedule	Risk Group 4 Biological Agents which can cause severe / lethal disease, easily transmitted and of high risk to the individual and the community. These agents have the potential to be weaponised.	14
Third Schedule	Risk Group 2 Biological Agents that need special attention in large scale production.	3
Fourth Schedule	All Risk Group 2 Biological Agents (including those in Third Schedule) which cause disease in humans.	250+
Fifth Schedule	Microbial toxins that have the potential to be weaponised.	7

Source: MOH Biosafety website <http://www.biosafety.moh.gov.sg/home/page.aspx?id=61> (accessed 11 Oct 2012)

The [List of Biological Agents and Toxins](#) classified under each of the BATA schedules can be accessed at MOH Biosafety website.

Biological agents that have been rendered non-infectious and unable to replicate itself under any condition are classified as **Inactivated Agents**, and pertain to biological agents in First and Second Schedule under BATA.

3.2.2 Approvals and Permits

Necessary approvals and permits, applicable to facilities for importing, possessing, producing in large scale, handling, and transferring and transporting biological agents and toxins in each Schedule are listed in Table 3.2.

Table 3.2 Summary of controls of the five Schedules in BATA

Requirement	First Schedule		Second Schedule	Third Schedule	Fourth Schedule	Fifth Schedule	Inactivated Agents	
	Part I	Part II					First Schedule	Second Schedule
Import Permit	√	√	√	√	√	√	√	√
Transshipment Permit	√	√	√	NA	NA	√	NA	NA
Approval to Possess	√	√	√	NA	NA	√	NA	NA
Approval to Produce	√	√	X	√	NA	NA	NA	NA
Special Approval to Handle	NA	NA	√	NA	NA	NA	NA	NA
Transfer Notification	NA	√	√	NA	NA	√	NA	NA
Certified Facility	√	√	√	NA	NA	NA	NA	NA
Protected Place	NA	√	√	NA	NA	√	NA	NA

√ - Required

X - Prohibited

NA – Not required

Source: MOH Biosafety website

http://www.biosafety.moh.gov.sg/home/uploadedFiles/Common/Schedule_Controls.pdf
(accessed 15 May 2013)

OSHE will assist researchers in the submission of documents to Regulatory Policy Branch (Biosafety Team) at MOH for the application of the approvals and notifications, except for import permits, described below.

a. Approval for Import

An import permit is required before importation into Singapore of any biological agents or toxins in the [List of Biological Agents and Toxins](#), including inactivated First and Second Schedule agents. However, the list is not exhaustive. Importation of other potential human pathogens may be prohibited. Please seek clarification in writing from Regulatory Policy Branch (Biosafety Team) at MOH for approval, with the detailed product information, if you are importing any biological materials that are not found in this list.

The application of import permit is done through the [Singapore Customs TradeNet System](#). Researchers intending to import any BATA agents and toxins may request the courier company engaged for the importation to apply for the import permit on their behalf. Please refer to the guidelines on [Import and Transshipment of Biological Agents and Toxins](#) in MOH Biosafety website.

Each import permit is valid only for the specific consignment of the biological agent or toxin.

When importation of inactivated agents of First and Second Schedules is involved, Regulatory Policy Branch (Biosafety Team) at MOH may request for the inactivation protocol and other related documents to validate the claim that the agent is inactivated. As such, the end users need to have supporting documentation on hand when such agents are to be imported.

b. Approval to Possess

An approval to possess is an approval granted by MOH to possess First, Second and Fifth Schedule biological agents and toxins, which is agent specific and is issued when the person who requires the biological agent or toxin has put in place adequate measures to contain the risk to public health and security posed by the biological agent or toxin.

- Protected Place: under the BATA, being gazetted as a protected place under the Protected Areas and Protected Places Act is a pre-requisite for the application for possession of First Schedule Part II, Second Schedule biological agents and Fifth Schedule toxins.
- Certified facility: in addition, the possession and use of First Schedule and Second Schedule biological agents also require a certified facility (typically a BSL3 facility), which has to be certified by a [MOH-Approved Facility Certifier \(AFC\)](#).
- Uncertified facility: work in an uncertified facility may be granted, on a case-by-case basis. An uncertified facility is a facility that is not certified as defined in the Biological Agents and Toxins Act but has met the requirements of the Ministry of Health to possess First Schedule biological agents and Fifth Schedule toxins.

Third and Fourth Schedule biological agents do not require an approval to possess.

c. Approval to Produce (Large-Scale Production)

Approval to produce is applicable only to First and Third Schedule biological agents when produced in large scale. Large-scale production is defined as the production by any person of the biological agent using equipment at a facility capable of producing in aggregate 10 or more litres of the biological agent at any one time.

For large-scale production of First Schedule biological agents, an application for approval to possess is required. The facility would need to meet the requirements to possess the biological agent before MOH will consider granting the approval for large-scale production of the biological agent.

For large-scale production of Third Schedule biological agents, an approval to possess is not required, and you may directly apply for an approval to produce.

Large-scale production is prohibited for Second Schedule biological agents.

d. Special Approval to Handle

A special approval to handle is only applicable to Second Schedule biological agents and is only granted by MOH under the following circumstances:

- i. The use of the Second Schedule biological agent is necessary for the public interest.
- ii. The person who requires the biological agent has put in place adequate measures to contain the risks to public health and security posed by the Second Schedule biological agent.

e. Transfer Notification

The transfer notification shall only apply to First Schedule Part II and Second Schedule biological agents, and Fifth Schedule toxins.

BATA requires that both the transferor (sender) and transferee (receiver) have a valid approval to possess the agent before effecting a transfer. The transferor shall ensure that the transferee has a valid approval to possess before he proceeds to transfer the biological agent or toxin.

In addition, the transferor has the responsibility to notify MOH of the proposed transfer by creating a notification of transfer via the [Biosafety I.T. Services](#) (BiosIS) E-services or send an e-mail to MOH (moh_biosafety@moh.gov.sg), if the transferor is not a registered user of the BiosIS E-services.

Under BATA, the transferee (receiver) shall notify **failure of receipt of transfer** to the MOH if he/she did not receive the consignment within 24 hours of the estimated time of receipt of the agent provided by the transferor (sender).

3.2.3 Transportation

The local transportation requirements for biological agents and toxins within Singapore are specified in the Biological Agents and Toxins Act 2005 and the [Biological Agents and Toxins \(Transportation\) Regulations 2005](#).

Public transportation including taxis and sending through mail is prohibited for all agents and toxins regulated under BATA.

In addition, Biological Agents and Toxins (Transportation) Regulations 2005 specifies transportation requirements for packaging, labeling of packages, and training of drivers that drive the conveyance in which any of the above agents are carried, applicable to:

- First Schedule biological agent;
- Second Schedule biological agent;
- Third Schedule biological agent in quantities aggregating 10 litres or more carried on any conveyance at any one time; *and*
- Fifth Schedule toxin.

Please refer to Section 9.1 of this manual for more details on the requirements.

3.2.4 Notifiable Incidents to MOH

Notifiable incidents to MOH for facilities handling First Schedule, Second Schedule and Third Schedule biological agents and Fifth Schedule toxins in accordance to BATA section 44 (c) are as follows:

- all confirmed or suspected infections or illnesses acquired by any member of the staff of the facility in the course of carrying out any activity involving biological agents or toxins at the facility;
- all adverse incidents involving biological agents that may potentially cause transmission of any infectious disease;

- all adverse incidents involving toxins;
- all loss, whether through theft or otherwise, of biological agents and toxins; *and*
- the destruction by the operator of the facility of any of his stocks of First Schedule (Part II) biological agents, Second Schedule biological agents and Fifth Schedule toxins.

3.2.5 Joint Control with Other Agencies

Some of the biological agents regulated under the BATA Schedules may also cause diseases in animals, and are termed as zoonotic pathogens. These agents are jointly regulated by MOH as biological agents under BATA and by Agri-Food & Veterinary Authority (AVA) of Singapore as veterinary biologics under the Animals and Birds Act (see Section 3.3).

If the biological agent regulated under BATA is genetically modified or is going to be used for genetic modification work, a proposal needs to be submitted to Genetic Modification Advisory Committee (GMAC) as specified in [Singapore Biosafety Guidelines for Research on Genetically Modified Organisms \(GMOs\)](#), for review and endorsement (see Section 3.5).

3.3 ANIMALS AND BIRDS ACT

The [Animals and Birds Act](#), administered by the Agri-Food & Veterinary Authority (AVA) of Singapore, is an Act for preventing the introduction into, and the spreading within, Singapore of diseases of animals, birds or fish; for the control of the movement of animals, birds or fish into, within and from Singapore; for the prevention of cruelty to animals, birds or fish; for measures pertaining to the general welfare and improvement of animals, birds or fish in Singapore and for purposes incidental thereto.

3.3.1 Laboratory Animals

All NUS animal holding facilities must be licensed by AVA to use animals for scientific purposes. In addition, the import, export and transshipment of laboratory animals are controlled by the Act and accordingly, only facilities licensed by AVA to use animals for scientific purposes may import and export laboratory animals.

3.3.2 Veterinary Biologics

In addition to laboratory animals, a number of veterinary biologics and pathogens are also regulated under this Act.

Veterinary biologics include “any viruses, serums, toxins and analogous products of natural or synthetic origin, including genetically modified organisms, diagnostics, antitoxins, vaccines, live micro-organisms, killed micro-organisms and the antigenic or immunizing component of micro-organisms intended for use in the diagnosis, treatment or prevention of diseases of animals and birds, or for purposes of research in animals or birds”.

A licence to possess and an import license are required for possession and importation of any of the veterinary biologics regulated by AVA.

The [List of Pathogens](#) (bacteria, virus, parasites and fungi) of veterinary biologics regulated by AVA is accessible at the AVA website. However, this list is not exhaustive. Thus, importation of other potential animal pathogens may be prohibited. The Import and Export Regulation Division in AVA should be consulted for importation of any potential animal pathogen which is not listed on this list.

Some of the veterinary biologics regulated by AVA under the Animals and Birds Act are also regulated as biological agents by MOH under the BATA. These require approvals from both regulatory agencies.

Researchers can seek assistance from OSHE to fulfill regulatory requirements pertaining to the use of AVA-regulated veterinary biologics or materials.

3.4 CONTROL OF VECTORS AND PESTICIDES ACT

The Control of Vectors and Pesticides Act, which is administered by the National Environment Agency (NEA) is applicable to researchers who wish to use vectors such as mosquitoes for research purposes. Under this Act, vectors are defined as “any insect, including its egg, larva and pupa, and any rodent, including its young, carrying or causing, or capable of carrying or causing any disease to human beings”.

An approval from NEA needs to be sought before importing (if applicable) and working with insects and rodents that are considered as vectors under this definition.

OSHE can be contacted for assistance in cases where researchers wish to use such insects or rodents.

Please note that the use of these insects and animals may also fall under the purview of AVA. In such cases approvals from both AVA and NEA need to be sought.

3.5 SINGAPORE BIOSAFETY GUIDELINES FOR RESEARCH ON GENETICALLY MODIFIED ORGANISMS

The [Singapore Biosafety Guidelines for Research on Genetically Modified Organisms \(GMOs\)](#) developed by the Genetic Modification Advisory Committee (GMAC) was first released in 2006.

3.5.1 Scope

The objective of the guidelines is “to ensure the safe containment, handling and transport of genetically modified organisms used in research and to provide a common framework for assessment and notification of research on GMOs”.

The scope of the guidelines cover experiments “that involve the construction and/or propagation of all biological entities (cells, organisms, prions, viroids or viruses, plants and animals) which have been made by genetic manipulation and are of a novel genotype and which are unlikely to occur naturally or which could cause public health or environmental hazards”.

As such, the requirements mentioned in the guidelines are applicable to and need to be followed by NUS researchers who are carrying out genetic manipulation work or using genetically modified organisms (GMOs) that fall under the scope of the guidelines. The [Singapore Biosafety Guidelines for Research on Genetically Modified Organisms \(GMOs\)](#) can be accessed from GMAC’s website.

3.5.2 Categories of Work

Under the guidelines, genetic modification experiments are classified into 3 categories as Category A, B and C. The types of experiments that fall under each category are:

Category A - Regulated experiments with significant risks

Category B - Notifiable experiments with low risks

Category C - Experiments with no significant risks

Category A experiments require IBC approval and GMAC endorsement while category B experiments require IBC approval and GMAC notification.

Category C experiments are exempted from GMAC notification. A list of experiments for which exemption has been granted as well as a list of exempted GMAC approved host/vector systems are given in the guidelines.

For more information, refer to Chapter 4 of the GMAC guidelines for the types of experiments that fall under each category.

3.5.3 Documentation

For work that falls under the scope of Category A or category B, PIs must fill in the [Proposal Form for Assessment of Genetic Manipulation Work](#) and submit to GMAC via IBC / OSHE.

GMAC endorsement is granted for 3 years. If PIs wish to continue the genetic modification work beyond 3 years for which the endorsement has been granted they need to apply for renewal by submitting the [Proposal Form for Extension of GMAC Endorsement](#) via IBC / OSHE.

OSHE will assist researchers in submitting documents to GMAC.

3.5.4 Transportation

GMAC guidelines describe the requirements for transportation including regulatory requirements and detailed requirements for containment based on risk level for:

- Genetically modified microorganisms
- Genetically modified animals
- Genetically modified plants
- Genetically modified arthropod and their pathogens

Please refer to Chapter 6 of the GMAC guidelines for details of transport and packaging.

3.5.5 Joint Controls with Other Agencies

For use or import of genetically modified biological agents or materials regulated by MOH, AVA or any other regulatory agency, GMAC endorsement must be sought first before applying for approval from relevant regulatory agencies and/or applying for import permit / licence.

3.6 GUIDELINES ON THE CARE AND USE OF ANIMALS FOR SCIENTIFIC PURPOSES

The guidelines on the care and use of animals for scientific purposes developed by the National Advisory Committee for Laboratory Animal Research (NACLAR) was first published in 2004. These national guidelines “establish the best practices in the use and care of animals for scientific purposes”, and “set out the responsibilities of all the parties involved in the care and use of animals for scientific purposes, in accordance with widely accepted scientific, ethical and legal principles”.

The guidelines “stipulate that all proposed use of animals for scientific purposes must be evaluated by an Institutional Animal Care and Use Committee (IACUC) in compliance with the Guidelines”.

Please refer to the [Guidelines on the Care and Use of Animals for Scientific Purposes](#) for more information.

INSTITUTIONAL / NUS REQUIREMENTS FOR WORK INVOLVING BIOLOGICAL MATERIALS

All Principal Investigators who plan to use biohazardous agents, genetically modified organisms (GMOs), animals and transgenic animals are required to complete and submit a project risk assessment before any new research project is implemented (unless exempted under the NUS Laboratory Occupational Safety & Health Certification Scheme); or when there are changes that may affect the safety and health aspects of the project or as and when required by the University.

Details of Project Risk Assessment submission are available through the Office of Safety, Health and Environmental (OSHE) website on [Research Safety Compliance Form Submission](#).

All risk assessment submissions for projects requiring grant funding are to be submitted to the respective Heads of Departments (HODs) / Deans for endorsement prior to their submissions to the IBC. For projects that do not require any grant funding (e.g. teaching activities, dissertation projects) risk assessments are approved by the HOD or his designate and do not need to be submitted to the IBC for approval. PIs can only commence work after their risk assessments have been approved.

All PIs are accountable for the inventory of the biohazardous agents in his/her lab and are responsible for ensuring safe operation of the laboratory.

For work within BSL3 / ABSL3 facilities, staff and students must comply with the requirements as defined in the NUS Safety Directive ([Directive No. 0703](#)) on *Staff and Students Working in BSL3 Facilities*.

For work with a combination of biohazardous materials and hazardous chemicals and/or radiation hazards such as radionuclides, please adhere to the corresponding guidelines in the [NUS Laboratory Chemical Safety Manual](#) and [NUS Laboratory Ionizing Radiation Safety Manual](#).

3.7 OVERSIGHT BY INSTITUTIONAL COMMITTEES

Institutional committees are the final authorities at the University level that ensure staff and students in NUS comply with institutional requirements. As such, for research PIs, approvals from appropriate institutional committees need to be obtained before funds will be released for new grant projects.

The three institutional committees that oversee work involving biological materials are:

- The Institutional Biosafety Committee (IBC)
- The Institutional Animal Care and Use Committee (IACUC)
- The Institutional Review Board (IRB)

3.7.1 Scope of the Institutional Biosafety Committee (IBC)

The use of biological agents, toxins of biological origin, animal and human tissues, genetically modified organisms (GMOs), animals, including transgenic animals, and plants, including transgenic plants that may potentially affect the safety and health of NUS staff and students, are under the purview of the IBC. Therefore, research projects that use biological materials require IBC approval prior to work commencement. For all research projects that require grant funding, endorsement from Head of Department / Director of Research Institutes need to be sought before submitting for IBC approval.

In addition, when PIs wish to use regulated biological agents / materials, GMOs or carry out genetic modification work that falls under Category A or B, IBC needs to be notified and where applicable, approvals need to be sought. All notifications or applications for approval to IBC need to be submitted through OSHE, who manages the administrative matters for IBC. The term “IBC / OSHE” will be used when IBC requirements are discussed in the following section.

3.7.2 Scope of Institutional Animal Care and Use Committee (IACUC)

IACUC is the institutional committee that oversees the care and use of animals for scientific purposes in NUS. IACUC ensures the housing and care of animals are provided in accordance to [Guidelines on the Care and Use of Animals for Scientific Purposes](#) by National Advisory Committee for Laboratory Animal Research (NACLAR), taking into consideration the relevant scientific, ethical and legal issues. All PIs who wish to carry out any work with vertebrate animals need to seek approval from IACUC and ensure that the animal experiments are carried out in strict accordance to IACUC-approved protocols.

For more details on IACUC and document submission to IACUC, please refer to information on [IACUC website](#).

3.7.3 Scope of Institutional Review Board (IRB)

The NUS Institutional Review Board (IRB) will review, approve and monitor the ethical aspects of all NUS research projects that involve human subjects and human tissues/cells/data.

For more information, please refer to [IRB website](#).

3.7.4 Overlapping Joint Oversight of Institutional Committees

PIs who wish to carry out animal work need to seek approvals from both IACUC and IBC while the use of material of human origin requires both IRB and IBC approvals.

3.8 INSTITUTIONAL RISK MANAGEMENT REQUIREMENTS *INCLUDING APPLYING FOR IBC APPROVALS / NOTIFICATIONS*

All Principal Investigators who plan to use biological agents, toxins of biological origin, animal and human tissues, genetically modified organisms (GMOs), animals including transgenic animals, and plants including transgenic plants are required to conduct risk assessment and to ensure that all necessary risk controls are in place. To conduct a risk assessment, please refer to Chapter 4. Chapters 5 – 12 discuss the necessary and required controls that need to be in place for handling biological materials.

All PIs are responsible for maintaining the relevant documentation related to the use of biological materials, including maintaining an inventory, where applicable, and ensuring safe operation of the laboratory.

3.8.1 PIs Certified under the Laboratory Certification Scheme

To comply with the [Workplace Safety and Health \(Risk Management\) Regulations](#) and to facilitate effective risk assessment and management, NUS has introduced the Laboratory Safety and Health Safety Management System Certification Scheme. The main requirement of this scheme is to perform risk assessments for all laboratory activities. Therefore, certified PIs must perform and document risk assessments (RAs) for all work involving potentially biohazardous materials. These RAs are to be signed and are legal documents under the WSH Act.

It is mandatory for PIs to notify IBC / OSHE if a certified PI wishes to start any of the following work:

- Use of a regulated biological agent / material by MOH, AVA, NEA, etc.
- Use of a genetically modified agent / material that falls under Category A or B of GMAC guidelines.

IBC / OSHE should be notified of such changes by submitting:

- A new application and any subsequent amendments of this approved protocol via the [integrated Online Research Compliance System](#) (iORC), if the work is a new research project
- *Amendment Form* ([Form OSHE/PI/F/06](#)) via a hard copy submission, if the new work is addition to an ongoing project that was previously submitted through Online Project Risk Assessment System (OPRAS)

Depending on the regulatory classification of the biological agent / material, additional documentation may be required. OSHE will notify the PI if this necessary.

As most national agencies require institutional approval before granting their approval / endorsements, IBC approvals will be issued if applicable.

3.8.2 New PIs / PIs not certified under the Laboratory Certification Scheme

Generally, when a new research PI joins NUS, a period between 6 months to 1 year is given to the new PI to adjust to institutional and national requirements before they undergo the Laboratory Certification Scheme. During this time, PIs are required to notify IBC / OSHE of all research involving potentially biohazardous materials, including regulated biological agents / materials through the iORC system. This system can be used to declare the biological work at the beginning of a project as well as when new biological work is to be commenced during the course of the approved project.

For non-certified PIs, IBC will review and issue an approval for new work as well as all amendments to these projects. If approvals / endorsements from national agencies are required, these IBC approvals can be used as institutional approvals.

Once these PIs are certified they can follow the procedure for certified PIs given under Section 3.8.1.

3.8.3 IBC / OSHE support for applications submitted to regulatory / endorsement agencies

The use of the following biological agents / materials requires IBC approval before application to the respective regulatory agencies:

- First and Second Schedules biological agents and Fifth Schedule toxins regulated by BATA
- Risk Group 3 veterinary biologics regulated by AVA
- Vectors regulated by NEA

Therefore, once IBC / OSHE is notified of the proposed use of these agents / material via the portals described in Sections 3.8.1 and 3.8.2 above, OSHE will work with the PIs in preparing the relevant documentation and assist with documentation submission to the regulatory agencies.

For genetic modification work that falls under category A or B of the GMAC guidelines, IBC / OSHE needs to be notified via the portals described in Sections 3.8.1 and 3.8.2 above. In addition, the [Proposal Form for Assessment of Genetic Manipulation Work](#) prescribed by GMAC also needs to be submitted to IBC / OSHE. OSHE will submit the form to GMAC for its review.

3.8.4 Requirements for other types of work done in combination with biological work

Researchers will have to work with a variety of materials and equipment that can pose chemical, ionizing radiation, non-ionizing radiation, physical, electrical, and/or ergonomic risks to themselves.

In such cases, depending upon the type of work, the following NUS manuals need to be consulted, together with the *NUS Laboratory Biorisk Management Manual* to know more about these risks and how they can be managed:

- [NUS Laboratory Chemical Safety Manual](#)
- [NUS Laboratory Ionizing Radiation Safety Manual](#)
- [NUS Laboratory Laser Safety Manual](#)
- [NUS Fire Safety Manual](#)
- [NUS Laboratory Ergonomics Manual](#)
- [Noise & Vibration Monitoring and Management Standards for NUS Workplaces](#)

3.9 PATHOGENS AND TOXINS

All research projects involving biological agents, toxins, plant pathogens and animal pathogens must be submitted to IBC for review prior to commencement of work.

PIs should be familiar with the national requirements for the possession, use, import, transshipment, transfer and transportation of biological agents and toxins that are known to be hazardous to human health, which are regulated by MOH as specified in the BATA. Please refer to Section 3.2 of this manual for details.

PIs should also be familiar with the national requirements for the importation, exportation and transshipment of laboratory animals, and possession and importation of any of the veterinary biologics regulated by the AVA as specified in Animals and Birds Act. Please refer to Section 3.3 of this manual for more details.

3.10 VETERINARY BIOLOGICS

Veterinary biologics is defined as any viruses, serums, toxins and analogous products of natural or synthetic origin, including genetically modified organisms, diagnostics, antitoxins, vaccines, live micro-organisms, killed micro-organisms and the antigenic or immunizing component of micro-organisms intended for use in the diagnosis, treatment or prevention of diseases of animals and birds, or for purposes of research in animals or birds.

Regulation of veterinary biologics is part of AVA's role in safeguarding the health of animals and birds, under the [Animals and Birds Act \(Chapter 7\)](#).

In order to possess veterinary biologics (veterinary vaccines and pathogens on the [AVA's Select List](#)), the applicant must first be a holder of either the Licence to Possess Veterinary Biologics (Commercial Purposes) or the Licence to Possess Veterinary Biologics (Research and Development Purposes).

NUS has been granted an institutional licence to possess Risk Group 2 (RG2) veterinary biologics on 1 April 2013 by AVA. The NUS institutional licence covers RG2 veterinary biologics in the [AVA's Select List](#), and includes organisms / AVA-registered vaccines / proteins / peptides / other genetic materials of RG2 veterinary biologics. This arrangement is only applicable to PIs located in the Kent Ridge campus.

More information on [NUS Institutional Licence to Possess Risk Group 2 Veterinary Biologics](#) is available at OSHE website.

3.10.1 Identifying Risk Groups of Veterinary Biologics and the Requirements

- a. PI proposing to work with an infectious agent should review the [AVA's Select List](#) of pathogens to determine if the infectious agent to be handled in the proposed research is regulated by the AVA.
- b. If the infectious agent is regulated by AVA and is designated as a RG2 infectious agent, it is a veterinary biologic covered under the NUS institutional licence and will be subjected to the requirements stipulated by AVA for this licence.
- c. The risk group is denoted as the first number appearing in the Product Code.
- d. If the infectious agent is not on the [AVA's Select List](#) but has the potential for zoonotic transmission, the PI must complete and submit an AVA form "Evaluation of Risk Posed by the Importation of Animal Pathogens into Singapore" to OSHE for further evaluation by AVA. Please consult OSHE staff if in doubt.
- e. If the veterinary biologic is a Risk Group 3 veterinary biologic, PI shall submit an application to AVA for a Licence to Possess Veterinary Biologics (Research and Development Purposes).

3.10.2 Registration to Possess Risk Group 2 Veterinary Biologics in NUS

Current AVA Licence Holders

The AVA Licence to Possess Veterinary Biologics (LPVB) held by individual PIs for their RG2 veterinary biologics shall be superseded by the NUS institutional licence. These PIs must now register their possession of the RG2 veterinary biologic with the OSHE in accordance with the terms and conditions of this NUS institutional licence.

New Registration

Any PI in NUS (located in Kent Ridge campus) who intends to import / acquire a biological agent should refer to the [AVA's Select List](#) to determine whether the biological agent is regulated by AVA. If it is a RG2 veterinary biologic regulated by AVA, PI must register their intention to possess the RG2 veterinary biologic with OSHE.

a. Submission of Documents

PIs must submit the following documents to OSHE:

- [Registration Form to Possess Risk Group 2 Veterinary Biologics](#) (OSHE/F/BS/02)
- Experiment-based Risk Assessments
- Standard Operating Procedures

The experiment-based Risk Assessments and Standard Operating Procedures shall include the following:

- Importation/exportation (receipt, unpacking, handling of incoming veterinary biologics, packaging requirements that meets IATA regulations for air transport, transfer to only licensed/NUS-registered laboratories, etc.)
- Transportation (packaging and transportation procedures)
- Access control to the laboratory and storage of the veterinary biologics (biosecurity)
- Culture (RG2 veterinary biologics shall be handled in BSL2 facilities with BSL2 safe practices)
- Decontamination (bench top, BSC, cultures/veterinary biologics, equipment, etc.)
- Waste disposal
- Training
- Medical surveillance (enrollment in Occupational Health Programme and vaccination if applicable)
- Pest control programme
- Emergency management:
 - Incident response (spills inside and outside of BSC)
 - Potential personnel exposure
 - Fire
 - Loss of electrical power
 - Loss of HVAC
 - BSC malfunction and dysfunction
 - Incident reporting, investigation and corrective actions

b. OSHE Inspection / Audit

- OSHE auditors will conduct an inspection/audit of the laboratory facilities where the RG2 veterinary biologics will be stored and handled.
- Registered PIs & laboratories shall be subjected to annual inspections/audits by OSHE auditors, as required by the terms of the AVA licence to NUS/OSHE.
- The PI will be given 1 month to rectify any deficiencies found during the inspection/audit.

c. Approval

- After the risk assessment is approved and any non-compliant issues found during the inspection/audit are cleared, OSHE will issue a registration approval.
- The approval is subjected to yearly audit inspection by OSHE.
- This approval will be required for any import permit needed to bring the agents into Singapore.

d. Termination

- If the PI has completed the research project with the registered RG2 veterinary biologics, and no longer wishes to keep any stocks of them, the PI must notify OSHE to terminate the registration.
- The PI must destroy all the RG2 veterinary biologics, and notify OSHE on the date of destruction.

3.10.3 Importation

- For imports of the RG2 veterinary biologics from a commercial source or from a collaborator, OSHE must be notified prior to import so that any necessary supporting documents can be issued.
- A licensed courier can then be contacted and arrangements for the import permit and shipping be finalized.
- Copies of all permits and shipping documents must be sent to OSHE. The PI shall also inform OSHE of the date when the RG2 veterinary biologics are received.

3.10.4 Transfer

- If the PI receives a request to transfer the registered RG2 veterinary biologics to another PI in NUS, the PI must notify OSHE of the intended transfer.
- If the PI receives a request to transfer the registered RG2 veterinary biologics to a non-NUS PI in Singapore, the PI must ensure that the recipient has the licence to possess the veterinary biologics prior to transfer.

3.10.5 Transportation of RG2 Veterinary Biologics

- The RG2 veterinary biologics are considered “regulated biological materials” and the guidelines for transport of regulated materials in Section 9.1 of this manual shall be followed.

b. Receipt of packages:

- Packages containing RG2 veterinary biologics should only be transported by couriers licensed in Singapore.
- All packages containing RG2 veterinary biologics should be inspected and opened in a designated BSC in an approved facility by trained personnel.
- Any leakages must be treated with the appropriate spill response, and an incident/accident report must be submitted to OSHE within 24 hours.

3.10.6 Records

The PI should ensure that the following documentation is kept, either in electronic or hardcopy versions, for at least three years:

- Risk assessments (RAs)
- Standard Operating Procedures (SOPs)
- Biological safety cabinet (BSC) testing and repair reports
- Training records for all staff and students
- Access authorizations
- Changes in personnel
- Transport: shipping documents, import permits, export permits
- Inventory of veterinary biologics
- If autoclave is used to decontaminate biohazardous materials: autoclave user log, validation testing records (biological indicators), pressure vessel certification, repairs

3.11 ANIMALS

Husbandry and research use of laboratory animals may present physical, chemical, biological/zoonotic, and radioactive risks. Please refer to the [NUS Occupational Health and Safety Manual for Personnel with Research Animal Contact](#) for work involving direct or indirect contact with laboratory animals.

a. **Animal work involving infectious agents or biological toxins**

For biosafety requirements of animal work, please refer to Section 4.5 of this manual.

b. **Animal work involving hazardous chemicals (chemical toxins, carcinogens, pharmaceuticals)**

For research experiments involving animals and chemicals that could pose a health hazard, the requirements outlined in the [NUS Laboratory Chemical Safety Manual](#) are applicable.

c. **Transgenic animals**

Experiments involving the generation of transgenic animals in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived there from, into the germ-line (e.g. transgenic rodents) must be approved by and conducted in accordance with the IACUC and GMAC (refer to GMAC Guidelines Appendix 12). Special care is to be taken to prevent accidental release of transgenic animals into the environment.

3.11.1 Institutional Approvals and Document Submission

All animal work in NUS requires approval from both the IACUC and IBC.

For certified and non-certified PIs, the Animals Procedure B form for new research grant projects that involve animal work are required to complete and submit through the [integrated Online Research Compliance System \(iORC\)](#). This form will be routed to both the IBC / OSHE and IACUC for review before approval will be granted from both institutional committees.

Any revisions to animal work for original projects submitted through [iORC](#) shall be amended using the amendment option in the iORC.

For PIs who wish to make a revision / amendment to research projects with animal work that were approved prior to the implementation of iORC, necessary forms have to be submitted in hardcopy to IACUC for approval and *Amendment Form* ([Form OSHE/PI/F/06](#)) to OSHE for IBC approval.

3.11.2 Risk Consideration for Administering Hazardous Materials into Animals

Working with animals presents some unique risks that may not be associated with working with other types of biological materials. These risks will not be limited to the actual use of animals but will also be associated with materials that are administered into animals. These could most commonly be biological material (human and animal pathogens, biological toxins, human tissues, cell lines, etc.), chemicals (chemical toxins, carcinogens, cytotoxic drugs, etc.), or radioactive material or a combination of such material. In addition, risks may be posed not only to researchers working with animals but also to animal facility husbandry staff. Therefore, please refer to [NUS Occupational Health and Safety Manual for Personnel with Research Animal Contact](#) in addition to this manual for animal work.

3.11.3 IBC / OSHE Support for Applications Submitted to Regulatory / Endorsement Agencies

Animal work that involves the administration of a regulated biological agent / material requires the relevant regulatory approvals / notifications prior to commencement of work. Additionally, if the transgenic animals used or created fall under the scope of Category A or B of GMAC guidelines, GMAC endorsement needs to be sought. The procedures are outlined in Section 3.8.3 of this manual.

3.11.4 Role of Comparative Medicine (CM)

Comparative Medicine (CM) manages the animal facilities within NUS. Therefore, when researchers are housing their animals in CM-managed facilities, CM requirements need to be followed. More information relating to CM and their requirements can be found in the [NUS Occupational Health and Safety Manual for Personnel with Research Animal Contact](#).

All NUS animal holding facilities and the importation of laboratory animals for scientific purposes that are managed by CM are licensed by AVA. Therefore, researchers who wish to import or export laboratory animals must consult IACUC and CM for proper procedures to ensure compliance.

3.12 PLANTS

Research experiments with genetically engineered plants, genetically engineered plant-associated microorganisms should adhere to the GMAC guidelines. The plant house biosafety requirements can be found in Appendix 10 or 11 of the GMAC guidelines.

3.13 HUMAN SUBJECTS / HUMAN TISSUES

All research projects involving the use of human subjects or human tissues must be submitted for review by the NUS Institutional Review Board (IRB) on the ethical use of human subjects. Refer to the [IRB](#) website for guidelines on application. For biosafety requirements, please refer to Section 4.4.2 of this manual.

3.14 GENETICALLY MODIFIED ORGANISMS (GMOs) & RECOMBINANT DNA EXPERIMENTS

For projects which involve genetic manipulation or the use of on genetically modified organisms (GMOs), the [Singapore Biosafety Guidelines for Research on Genetically Modified Organisms \(GMOs\)](#) by the Genetic Modification and Advisory Committee (GMAC) are to be adhered to. Please refer to Section 3.5 of this manual for full details.

3.15 TRAINING

The NUS administration has made it mandatory for all staff and students performing research work in laboratories to undergo institutional safety trainings that are applicable to them. OSHE is the provider of training under the University's [Structured Safety Training System \(SSTS\)](#). The safety trainings are available through e-learning in Integrated Virtual Learning Environment (IVLE) or via classroom training. Please refer to the [Training Registration Information](#) for information on OSHE courses available. Please refer to [Instructions to Complete the Safety Training through IVLE](#) for step-by-step guide on how to register and complete training through IVLE.

3.15.1 Biological Safety (OSHBIO01) & Biological Safety Refresher (OSHBIO02)

Biological Safety (OSHBIO01) training is compulsory for the following individuals involved in research work that handle biological materials:

- **Principal Investigators**
Although PIs may not be actively working in the laboratory, it is compulsory for new PIs who are not certified under the NUS Laboratory OH&S Certification Scheme (non-certified PIs) to undergo this training as there is a section on national and institutional / NUS requirements related to biological work which every PI must be aware of.
- **All laboratory and technical staff**
This includes Research Fellows (RFs), Research Assistants (RAs), Laboratory Managers, Laboratory Executives, Laboratory Technicians (LTs) and any other relevant staff.
- **All students doing research projects**
This includes postgraduate research students, undergraduate students doing research work or projects under the Undergraduate Research Opportunities Programme in Science (UROPS), undergraduate students doing their final year project (FYP).
- **Staff and students working in non-NUS organizations**

This includes NUS staff and students conducting research work or projects in A-STAR institutes, hospitals, industrial attachments, etc.

For non-NUS PIs / collaborators / attachment students or staff working temporarily in NUS laboratories, NUS PIs should evaluate their biological safety training needs. Contact OSHE staff (oshosk@nus.edu.sg) to obtain access for persons without NUS e-mail accounts.

The **Biological Safety Refresher (OSHBIO02)** training is required once every two years for the length of time the staff and students are working with biological materials. This Biological Safety Refresher (OSHBIO02) training is compulsory for all PIs who are certified under the NUS Laboratory OH&S Certification Scheme (certified PIs).

Instructions on Biological Safety Training and Biological Refresher Safety Training are available via e-learning through the IVLE (see Section 3.15). There is an open-book assessment at the end of the training, and a certificate will be issued within a week upon the successful completion of each course.

3.15.2 General Laboratory Safety (OSH0001)

Operational Associates who work in laboratories are recommended to attend OSHE's **General Laboratory Safety (OSH0001)** training course. This is a classroom training course which requires pre-registration and is conducted yearly. For more details, please refer to OSHE's [Structured Safety Training System](#) webpage.

3.15.3 Other OSHE Safety Trainings

Some other relevant Safety Training as listed below.

E-learning through the IVLE:

- Chemical Safety (OSHCHM01)
- Radiation Safety (Ionizing) (OSHRAD01)
- Laser Safety (OSHRAD02)

Classroom learning:

- Risk Management for Laboratories
- Fire Safety Education Course

Please refer to the [Structured Safety Training System \(SSTS\)](#) for the list of institutional safety courses available.

3.15.4 Responsible Care and Use of Laboratory Animals (RCULA) & Responsible Care and Use of Fish (RCUF)

All staff and students caring for, and using live vertebrates for scientific purposes at NUS must be educated, trained and qualified to use animals in a humane and ethical manner.

All NUS staff and personnel intending to handle live vertebrates must complete the following training courses which are provided by IACUC, depending on the type of live animals to be handled.

- a. Responsible Care and Use of Laboratory Animals (RCULA) conducted by Comparative Medicine (CM), for the use of common laboratory mammals

- b. Responsible Care and Use of Fish (RCUF) conducted by CM and Department of Biological Sciences, for the use of fish for scientific purposes

These training courses will give personnel a general understanding on the regulations and the responsibility in using animals for scientific purposes. Successful participants will be awarded certificates. The serial number on the certificates will be required when submitting protocols for IACUC review.

Information on these trainings including course schedules, fees, etc., is available at the [IACUC](#) website.

3.16 NUS OCCUPATIONAL HEALTH PROGRAMME

The NUS Occupational Health (OH) Programme provides a structured system to address the occupational health needs of NUS staff and students. Its purpose is to protect and promote the health of all personnel in the workplace.

The NUS OH Programme is a collaborative effort of the following offices / departments / personnel:

- **OSHE** is the general administrator of this programme by providing policies, standards and guidelines.
- **Occupational Health Clinic** with its Senior OH Physician, Resident Physician and OH Nurse conduct medical surveillance, treatment of work-related conditions and fitness to work requirements.
- **University Health Centre (UHC)** provides primary healthcare for staff and students
- **Office of Human Resources (OHR)** and **Registrar's Office (RO)** are the administrators for insurance policies for staff and students, respectively.

Principal Investigators (PIs) & Managers are responsible for identifying the occupational health needs of staff and students under their supervision, who works with materials of animal and human origin and infectious agents. Please refer to the [Occupational Health Services](#) section in the OSHE website for information on the type of medical surveillance available and the relevant forms. These include medical surveillance prescribed under legislative requirements and those required under institutional requirements.

An [Authorisation Form for Occupational Health Services](#) needs to be submitted OH Clinic first to request for any OH services at NUS.

3.16.1 Medical Surveillance for Personnel in Contact with Animals

Medical surveillance is applicable not only for NUS staff and students that are directly working with animals (live or dead) but also for those who would be indirectly exposed to animals by their tissues or fluids, cages and bedding, e.g. animal husbandry staff in animal holding facilities at NUS.

To enroll in this programme, personnel are required to complete the [NUS Animal Work Health Questionnaire](#) and submit it to the OH clinic. The OH physician will review the questionnaire and decide whether the person is fit to work with animals or whether further medical evaluation is needed (determined on a case-by-case basis). Selected personnel will require an animal work medical examination.

Minimally, all personnel who may come in contact with animals are required to be vaccinated against tetanus prior to starting work. After the initial vaccination, a booster dose is recommended every 10 years thereafter to maintain protective antibodies against tetanus.

Other medical surveillance requirements will depend on the type of risks involved or type of animal contact (e.g. personnel who work with non-human primates, those who work with stray animals, husbandry staff taking care of animals in CM facilities, etc.). These additional medical surveillance requirements will be decided by the OH physician based on the assessment of risks involved and evaluation of the [NUS Animal Work Health Questionnaire](#).

An OH Serial Number will be issued when the NUS personnel has enrolled in the OH Programme, and updated in the IACUC Register of Animal Workers.

Please refer to [NUS Lab Animal Work Occupational Health Programme](#) for detailed guidance on procedure to enroll for the OH Programme, medical surveillance for personnel in contact with laboratory animals and certification of fitness.

3.16.2 Medical Surveillance for Personnel Handling Materials of Human Origin

Hepatitis B vaccination is required for personnel who have a potential for exposure to materials of human origin (human blood, tissues, body fluids, cell lines, etc.) from both commercial and non-commercial sources. If the work done requires this vaccination, personnel will first be screened for antibodies against Hepatitis B prior to work. If the antibody levels are below the acceptable levels, a vaccination that consists of three doses or a booster will be given. Post-vaccination screening would be done to determine whether the antibody levels have reached to acceptable levels. Once deemed to be satisfactory, re-screening would be required every 5 years thereafter.

3.16.3 Medical Surveillance for Personnel Working in BSL2 / ABSL2 and BSL3 / ABSL3 Laboratories

Personnel who work in BSL2 / ABSL2 laboratories, especially those who work with Risk Group 2 biological agents in relation to laboratory work and/or animal work, should enroll in the medical surveillance programme before starting the work (for more information on Biosafety Levels and Risk Groups of biological agents, please refer to Chapter 4). The types of medical surveillance and the frequency of surveillance will depend on the scope of work and the types of agents to be handled in the laboratory and/or animal work.

Medical surveillance is mandatory for personnel working in BSL3 / ABSL3 laboratories to assess his/her fitness to work with infectious disease agents and/or animals before the start of work, annually thereafter, and finally at exit to identify potential ill-health following the end of the research project. Specific serological tests and vaccinations may be required.

An [NUS Biological Agents Work Medical Assessment Form](#) needs to be submitted to OH Clinic for medical assessment.

Please refer to [Medical Surveillance Requirements for Personnel Working in BSL3 and BSL2 Laboratories](#) for detailed information.

3.16.4 Respiratory Protection Programme

The [NUS Respiratory Protection Programme](#) requires that all user undergoes a fit test, spirometry and a medical examination to ensure that user selects the right-sized respirator, and is also medically fit to use the respirator. Restrictions may be imposed in certain medical situations to protect the health of the user.

The PI is responsible for providing appropriate personal protective equipment (PPE), ensuring that the personnel undergo fit-testing for respirators if the risk assessment for the procedures or the emergency response to spills calls for respirators. Staff and/or students should also receive information and instructions on proper usage, care of and disposal of such respirators.

Personnel working in BSL3 / ABSL3 facilities will require respiratory protection.

Please refer to [NUS Respiratory Protection Programme](#) for more details.

3.16.5 NUS Immunisation and Investigation Waiver Form

Personnel may elect to opt out of vaccination and medical surveillance services provided by the University. In such cases, an [NUS Immunisation and Investigation Waiver Form](#) should be completed by the concerned personnel.

3.16.6 Occupational-Related Disease, Illness or Infection

Medical assessment shall be done at the UHC or the OH Clinic during office hours, or at the Emergency Department of hospitals or outpatient clinics after office hours. Personnel working in a BSL3 facility should bring along their Medical Contact Card which has the contact information of their supervisor and the OH Physician.

In the event of an exposure to a hazardous agent resulting in a possible infection, allergy, disease or illness, the PI/manager shall ensure that:

- a. The staff/student is sent for medical assessment at UHC during office hours and to the Accident & Emergency Units of a hospital after office hours.
- b. A report is submitted to OSHE via the online [NUS Accident / Incident Reporting System \(AIRS\)](#).

Once the report is submitted, the OH physician together with OSHE, will investigate the accident, make recommendations and provide assistance in the rehabilitation of the exposed personnel.

3.16.7 Mandatory Occupational Health Controls

Additional mandatory OH controls are listed below:

a. Statutory Medical Examination Requirements in NUS

Under the Workplace Safety and Health (Medical Examinations) Regulations 2011, workers who are exposed to the hazards listed in [The Schedule](#) in the regulation are required to undergo mandatory specific pre-placement and periodic medical examinations called statutory medical examinations.

Please refer to [Statutory Medical Examination Requirements in NUS](#) which describes the coverage, periodicity and the conduct of statutory medical examinations for NUS staff and students pertaining to

hazards that require Statutory Medical Examinations.

b. Radiation Medical Examination (Ionizing & Non-Ionizing)

Laser (Non-Ionizing) Radiation Medical Examination is required for the submission of [N3](#) Licence form to NEA prior to work with lasers or laser equipment. The University staff and students may proceed to UHC with their staff cards or student matriculation cards for an appointment with the ophthalmologist for a detailed eye assessment.

Ionizing Radiation Medical Examination is required for the submission of [R1](#) Licence form to NEA prior to the issuance of licence for work with radioactive materials. The University staff and student may contact OH Clinic for Radiation Medical Examination.

Please refer to [Radiation Medical Examination \(Ionizing & Non-Ionizing\)](#) for details on these medical examinations.

3.17 UNIVERSITY LABORATORY COMMISSIONING / DECOMMISSIONING

3.17.1 Laboratory Commissioning

All laboratory designs for new or renovated laboratories and animal facilities in NUS require the approval of the Joint Safety Review Group (JSRG), jointly chaired by OSHE, OED staff, faculty safety officer or safety coordinator. The NUS [Laboratory Design Standard](#) provides guidelines on how laboratories involved in biological work should be designed and how safety equipment related to biological safety should be located. Please refer to [Laboratory Commissioning Checklist](#) for information on laboratory commissioning.

PIs must complete the [Laboratory Commissioning Notification & Verification Form](#) which should be verified by the Faculty Safety & Health Officer.

3.17.2 Laboratory Decommissioning

All PIs who are decommissioning a laboratory or laboratory area prior to leaving the University, relocating to another University laboratory, or renovating their laboratory, are required to follow the procedures described in [Laboratory Decommissioning Procedures](#).

PIs must complete the [Laboratory Decommissioning Notification & Verification Form](#) which should be verified by the Faculty Safety & Health Officer.

3.18 AFTER OFFICE HOURS

- a. Carrying out experimental laboratory work after office hours should preferably be performed using a “buddy system”. Working alone should be avoided, in case anything adverse should happen and help at hand is required.
- b. If experimental work must be conducted after office hours, the PI or supervisor should be informed.
- c. Certain types of work may not be undertaken outside of normal working hours (Monday - Thursday 8.30 am – 6.00 pm, Friday 8.30 am – 5.30 pm), for example, working with highly toxic

chemicals or hazardous biological materials. The PI should identify the activities that cannot be performed outside of the normal working hours.

- d. If experiments are to be run unattended overnight, it should be accompanied with a note containing information of the biological / chemical hazards involved, name of researcher and his/her contact number in case of an emergency.
- e. Undergraduate students and attachment students should not be allowed to work alone after office hours. The NUS Safety Directive (Directive No. 0701) for "[Access to and Supervision of Undergraduates in Laboratories for Project or Research Work](#)" shall apply.
- f. It is recommended that documentation of all work done after office hours is maintained (i.e. log of personnel, date, time in/out, etc.).

CHAPTER 4 RISK ASSESSMENT AND RISK MANAGEMENT

Responsibility for biosafety exists at all levels and is shared throughout the University. The NUS Biosafety Programme is administered to establish procedures for the safe use of biohazards and for compliance with all applicable regulations (please refer to Chapter 2 Biosafety Programme Administration). The PIs and all lab personnel who perform work with biohazards are the most important component of the biosafety programme, as they must incorporate the biosafety requirements and safety precautions into all facets of their work.

The PI is ultimately responsible for safety within the laboratory. An integral part of this responsibility is to conduct a risk assessment of proposed work to identify potential hazards and to adopt appropriate safety procedures before initiation of the experiments (risk management). Properly conducted, risk assessment can help prevent accidents/incidents as well as exposure to biohazards and minimize the potential for laboratory acquired infection and injuries.

4.1 INTRODUCTION TO BIOLOGICAL RISK ASSESSMENT / RISK MANAGEMENT STRATEGY

A risk assessment and management strategy should contain the following components:

- *Identification* of hazards
- *Assessment* of risks and available control measures
- *Control* of risks through implementation of control measure
- *Monitoring* of controls to evaluate their effectiveness.

Refer to OSHE's document [NUS Occupational Health and Safety \(OH&S\) Management System Standard for Laboratories - Part B: Guidance Notes](#) for guidelines on performing hazard identification, risk assessment and risk control.

The risk assessment determines a final biosafety level which then determines the risk management elements of laboratory facilities, equipment, training, and supervision required for the activities to proceed. Different elements of risk management are discussed in detail in Chapters 5-11 of this manual. Refer to the relevant sections for consideration of various control measures.

A risk assessment and risk management strategy for a biological laboratory should consider five primary factors or 'five P's' in each aspect of laboratory work:

- Pathogen – hazardous biological agent (infectious micro-organisms including potential pathogens harboured in other biological material)
- Procedures – experimental manipulations and safe work practices
- Personnel – training, skills, habits and attitudes
- Protective equipment – safety equipment and protective clothing
- Place – laboratory design, laboratory animals facility

4.1.1 Pathogen / Biological Agent

Key considerations of risk assessment:

- Agent risk group classification for infectious micro-organisms (see Section 4.2)
- Routes of infection
- Infectious disease process
- Virulence, pathogenicity, quantity, concentration

- Incidence in community
- Presence of vectors
- Availability of preventative measures and effective treatment
- Involvement of genetic manipulation

For all other biological hazards that do not clearly fall under risk group 1 or an equivalent low risk classification (e.g. potentially infectious genetically-modified micro-organisms) and nucleic acid products, as well as micro-organisms that could pose a hazard to the environment (plants, animals, habitats), the agent-specific risk assessment should take into consideration the above properties inherent to the biological material in order to assign a class of risk.

For genetically modified organisms (GMOs), the risk assessment should take into consideration the hazards arising from each of the elements used for construction of the GMO:

- the recipient micro-organism
- the donor micro-organism
- the inserted genetic material (originating from the donor organism)
- the vector
- the resulting GMO

Special attention should be given to vectors, nucleic acid products and special proteins such as:

- Replication-defective vectors that are infectious for mammalian cells
- Replication-competent vectors that are infectious for mammalian cells
- Nucleic acid material encoding oncogenes, growth factors and cytokines
- Fusion proteins (e.g. HIV TAT- or HSV VP22-fusion proteins)

Some examples of risk assessments and classifications are shown in Section 4.4.

Key elements of risk management:

- Appropriate biosafety level (Section 4.3-4.4)

4.1.2 Procedure

Not only should risk factors inherent to the biological material be considered, factors associated with the type of operations/manipulations should be examined as well.

Key considerations of risk assessment:

- Aerosol risk: sonicating, centrifuging, homogenizing, cell sorting, blending, shaking, etc.
- Percutaneous risk: needles, syringes, glass Pasteur pipettes, scalpels, cryostat blade/knife, animal bites/scratches, etc.
- Splash/splatter risk: pipetting, microbial loop, etc.
- Ingestion risk: mouth pipetting, eating, drinking, smoking
- Scale of the activity (e.g. lab vs field studies, test tube vs bioreactor)
- Concentration (e.g. clinical specimens vs cultures)
- Type of work (e.g. in vitro , in vivo , challenge studies, work with laboratory animals, non-standardized manipulations)
- Transfer/transportation of biological materials (e.g. transfer from incubator to BSC, transportation of patient's samples from hospital to lab)

Key elements of risk management:

- Written set of standard operating procedures (SOPs) with safety practices incorporated
- Adherence to basic biosafety principles and appropriate risk controls
- Good lab practices
- Label labs, areas, and equipment housing Risk Group 2 or higher agents
- Conduct lab inspections to review practices and containment equipment
- Use trial experiments with non-infectious material to test new procedures/equipment

4.1.3 Personnel

Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of co-workers are prerequisites for a laboratory staff in order to reduce the inherent risks that attend work with hazardous agents. In conducting a risk assessment, the Principal Investigator should identify any potential deficiencies in the practices of the laboratory workers and ensure that laboratory workers have acquired the technical proficiency in the use of microbiological practices and safety equipment required for the safe handling of the agent, and have developed good habits that sustain excellence in the performance of those practices.

It is also important to recognize that individuals in the laboratory may differ in their susceptibility to disease. Some of the conditions that may increase the risk of an individual for acquiring a laboratory-acquired infection (LAI) include preexisting diseases, medications, compromised immunity, and pregnancy or breast-feeding that may increase exposure of infants to certain agents.

Key considerations of risk assessment:

- Susceptibility to disease (neoplastic disease, infection, immunosuppressive therapy, age, race, sex, pregnancy, surgery, preexisting medical conditions, history of allergies and asthma)
- Immunization
- Post-exposure prophylaxis
- Attitude toward safety
- Knowledge, skills and experience
- Open wounds, non-intact skin, eczema, dermatitis

Key elements of risk management:

- Safety training
- Prior work experience with biohazards
- Demonstrated proficiency with techniques
- Prompt reporting of all exposure incidents, near misses, as well as signs and symptoms of related disease to PI and OSHE/UHC
- Investigation/review of incidents/spills, etc. to prevent future occurrence

4.1.4 Protective Equipment

Key considerations of risk assessment:

- Protection for exposure from aerosols, dust, droplets/splatter and sharps

Key elements of risk management:

- Safety equipment
 - Biological safety cabinets
 - Chemical fume hoods
 - Safety centrifuges and buckets

- Mechanical pipettes
- Training in proper use of safety equipment
- Personal protective equipment (PPE):
 - Respirators – N-95, PAPR etc.
 - Face (eye, nose, mouth) protection – mask and safety glasses, or face shield
 - Solid-front gown or lab coat
 - Gloves
- Training in proper use of PPE

4.1.5 Laboratory Facility

Key considerations of risk assessment:

- Risk group/biosafety level requirements
- Aerosol risk
- Accidental release of pathogen
- Use of laboratory equipment such as centrifuge, cell sorter, HPLC, etc.

Key elements of risk management:

- Basic lab facilities – door, sink, surfaces easily cleaned, eyewash, emergency showers
- Restricted access
- Labels
- Containment laboratory with directional airflow

It may be useful to determine the specific tasks with distinct objectives or equipment usage involved in an experiment before attempting to identify the hazards associated with each task. Please refer to examples given in [NUS Occupational Health and Safety \(OH&S\) Management System Standard for Laboratories - Part B: Guidance Notes](#).

Use the table to assist you in tabulating the risks and controls of your experiments. The relevant information can then be extracted out for submission of the NUS project-based risk assessments (see Section 4.7).

4.2 CLASSIFICATION OF BIOLOGICAL AGENTS

Biological agents are classified into Risk Groups based on their relative risks in different countries. Risk Group classifications are used in the research environment as part of a comprehensive biosafety risk assessment.

4.2.1 World Health Organization (WHO) Risk Group Classification

WHO recommends that each country classifies the agents in that country by Risk Group based on pathogenicity, modes of transmission, host range of the organism, and local availability of effective preventive measures and treatments. These may be influenced by existing levels of immunity, density and movement of host population, presence of appropriate vectors and standards of environmental hygiene.

Risk Group 1 (no or low individual and community risk)

A micro-organism that is unlikely to cause human disease or animal disease.

Risk Group 2 (moderate individual risk, low community risk)

A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventative measures are available and the risk of spread of infection is limited.

Risk Group 3 (high individual risk, low community risk)

A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

Risk Group 4 (high individual and community risk)

A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

The Risk Group classification of infectious agents varies from country to country. An agent classified into Risk Group 2 in one country may be classified as Risk Group 3 in another.

Please refer to the American Biological Safety Association (ABSA) online [Risk Group Database](#) search function that returns information of the risk grouping of a hazardous agent in various countries. Another useful reference is the [Pathogen Safety Data Sheets and Risk Assessment](#) by Public Health Agency of Canada.

4.2.2 Risk Groups and BATA Schedules

Under the present BATA, biological agents and toxins are classified into five schedules summarized in the table below. The 5 Schedules in BATA differentiates between higher Risk Group and lower Risk Group biological agents, those with potential to be weaponised and different national requirements needed for the possession, handling, working with and transportation of the biological agent / toxin.

The [List of Biological Agents and Toxins](#) classified under each of the BATA schedules can be found in the website of the MOH Biosafety section.

Table 4.1 Description of the 5 Schedules in BATA

Schedule Classification	Risk Group	Description of schedule
Schedule 1 (part 1)	3	Potential to cause serious disease which is high risk to individual
Schedule 1 (part 2)	3	(1) Potential to cause serious disease which is high risk to individual (2) Potential to be weaponised
Schedule 2	4	(1) Can cause severe/lethal disease, high risk to individual and community (2) Potential to be weaponised
Schedule 3	2	(1) Can infect humans (2) Need special attention in large scale production
Schedule 4	2	Can infect humans
Schedule 5	-	Microbial toxins with potential to be weaponised

NB. Schedule 1 is separated into part I and part II based on their potential to be weaponised.

4.2.3 Use of Biohazards in Combination with Other Hazardous Substances

Some experiments require the use of a combination of biohazards (such as an infectious agent) and chemical and/or radiological hazards such as drugs or radionuclides. Considerations should also be given as to the nature of these hazards during classification. Please refer to the corresponding manuals – [NUS Laboratory Chemical Safety Manual](#) and [NUS Laboratory Ionizing Radiation Safety Manual](#) are applicable.

4.2.4 Experiments with Animals and Biohazardous Substances

Animal experiments may involve animal pathogens or zoonotic pathogens (agents able to infect both humans and animals). The risk grouping of animal and zoonotic pathogens is guided by the same principles as described above.

In animal biosafety facilities, one hazard not normally present in biological laboratories is the route of transmission via animals. Dander, hair, aerosols (e.g. from sneezing and coughing from animals) and dust from urine or faeces – which can be inhaled, ingested or transmitted to mucous membranes or open wounds – are some hazards all people having access to animal facilities with infectious agents are exposed to. Animal husbandry, interventions and procedures bear specific risks such as aerosol and dust formation, animal scratches and bites and manipulations with sharps (e.g. inoculation with syringes) that should be addressed in the risk assessment.

For more details on risk assessments of animal work, refer to the [NUS Occupational Health and Safety Manual for Personnel with Research Animal Contact](#).

4.3 PRINCIPLES OF CONTAINMENT

4.3.1 Containment

The term "containment" is used in describing safe methods for managing biological hazards in the laboratory environment where they are being handled. Containment is required in order to reduce / eliminate exposure of laboratory workers, persons outside lab and the environment to potentially hazardous agents. It involves the application of a combination of three elements - laboratory practice and procedure, safety equipment and laboratory facilities, when working with biological hazards.

It can be accomplished through:

Primary Containment which is the protection of personnel and the immediate laboratory environment through good microbiological technique (laboratory practice) and the use of appropriate safety equipment.

and

Secondary Containment, the protection of the environment external to the laboratory from exposure to infectious materials through a combination of *laboratory facility design* and *operational practices*.

4.3.2 Laboratory Practices and Techniques

The most important element of containment is strict adherence to standard microbiological practices and techniques.

The use of aseptic techniques and other good microbiological practices achieves three very important objectives:

1. **OCCUPATIONAL HEALTH AND SAFETY:** The prevention of illness, disease or injury when working with biological hazards.
2. **ENVIRONMENTAL PROTECTION:** The prevention of contamination of the environment by the biological hazards being handled.
3. **PRODUCT PROTECTION:** The prevention of contamination of the work with biological agents from the environment.

The first and second objectives are of prime importance as regards to working safely whereas the third is a key element in relation to the quality of the research.

The Principal Investigator is responsible for ensuring that all personnel working with infectious agents or biological hazards are aware of potential hazards, and are properly trained and proficient in the practices and techniques required to handle such material safely. Lab personnel are the first line of defense for protecting themselves, others in the laboratory, and the public from exposure to hazardous agents.

Chapter 7 describes most of the standard recommended work practices and safe operating procedures common for microbiological laboratories. However, the list of practices is not exhaustive. Each laboratory should develop safe operating procedures specific to hazards that may be encountered, and which specifies practices and procedures designed to minimize or eliminate risks.

When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed. Laboratory personnel safety practices and techniques must be supplemented by appropriate facility design and engineering features, safety equipment and management practices.

4.3.3 Safety Equipment (Primary Containment)

Safety equipment includes biological safety cabinets, enclosed containers and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The principal device used for providing containment of infectious splashes or aerosols generated during work with biological material is the biological safety cabinet.

Chapter 5 describes common engineering controls used in the laboratory. Safety equipment also includes items for personal protection such as protective clothing (e.g. gowns, gloves), respirators, face shields, safety glasses or goggles (see Chapter 11). Personal protective equipment (PPE) is often used in combination with other safety equipment when working with biological agents and toxins. In some situations, protective clothing may form the primary barrier between personnel and the infectious materials.

4.3.4 Laboratory Facility Design

The primary function of the laboratory facility is to provide a physical environment in which work activity can be undertaken efficiently and safely. The design and construction of the facility constitute a secondary containment to provide varying degrees of barriers between the laboratory and the outside environment. The function of these barriers is to protect people working inside and outside the laboratory and to prevent the accidental release of micro-organisms into the environment in the event of a failure in a primary containment. Examples of secondary barriers include floors, walls and ceilings, air locks and self-closing doors, differential pressures between spaces (positive pressure and negative pressure designs to ventilation system), exhaust filtration, as well as devices for treating contaminated air, liquids and solids.

The design of a facility is important in providing a barrier to protect people working inside and outside the laboratory, and to protect people or animals in the community from infectious agents which may be accidentally released from the laboratory.

Facility design must be commensurate with the laboratory's function and the recommended biosafety level for the agent being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. Where there is a low risk of exposure, secondary barriers in these laboratories may include:

- separation of the lab area from public access
- availability of a decontamination facility (e.g. autoclave)
- hand washing facilities

Where there is a high risk of exposure to an infectious aerosol, higher levels of primary containment and multiple secondary barriers may be necessary to prevent infectious agents from escaping into the environment. These include:

- specialized ventilation systems for ensuring directional air flow
- air treatment systems for decontaminating the exhaust air
- controlled access zones
- airlocks as laboratory entrances
- or separate buildings or modules to physically isolate the laboratory

4.4 BIOSAFETY LEVELS

For each Risk Group of micro-organisms or risk classification of biological hazards there is a defined minimum set of control measures known as Containment Level or Biosafety Level (BSL) that reduces the exposure to an acceptable level for micro-organisms of that Risk Group.

There are four biosafety levels (BSL1, BSL2, BSL3 and BSL4). Each level of containment describes the microbiological practices, safety equipment and facility safeguards appropriate for the corresponding level of risk associated with handling a particular infectious agent. These levels, designated in ascending order, provide increasing levels of protection to personnel and the environment. Detailed descriptions of recommended practices, safety equipment and facility requirements for each BSL can be found in Chapter 3-5 of the WHO [Laboratory Biosafety Manual, 3rd Edition](#).

4.4.1 Biosafety Level 1 (BSL1)

Biosafety Level 1 is suitable for work done with well-characterized agents **not known to consistently cause disease in healthy adult humans**, and is of minimal potential hazard to laboratory personnel and the environment.

BSL1 represents a basic level of containment. Work is generally conducted on open bench tops using standard microbiological practices. These include wearing laboratory coats at all times and use of appropriate gloves for all procedures that may involve direct contact with biological materials in the laboratory. Personnel must wash their hands after handling biological materials, and before they leave the laboratory working areas. Open-toed footwear must not be worn in laboratories. Eating, drinking, smoking, applying cosmetics and handling contact lenses is prohibited in the laboratory working areas. Storing human foods or drinks anywhere in the laboratory working areas is prohibited.

Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

4.4.2 Biosafety Level 2 (BSL2)

Biosafety Level 2 (BSL2) is applicable to work done with agents associated with human disease but is of **moderate potential hazard** to personnel and the environment. Under NUS requirement, it is also applicable to work involving materials of human origin (human blood, tissues, body fluids, cell lines, etc.) from both commercial and non-commercial sources and other work with biological hazards classified by the PI or IBC as Risk Group 2.

- a. Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures or ingestion of infectious materials.
- b. BSL2 builds upon BSL1. Access to the laboratory is limited when work is being conducted.
- c. With good microbiological techniques, agents may be used safely on the open bench, provided the potential for producing splashes or aerosols is low.
- d. Primary barriers such as splash shields, face protection, gowns and gloves should be used as appropriate. Secondary barriers such as handwashing, eyewashes and waste decontamination facilities must be available.
- e. Extreme precautions are taken with contaminated sharp items.
- f. Procedures with high aerosol or splash potential must be conducted in primary containment equipment such as biological safety cabinets.

- g. Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists.

4.4.3 Biosafety Level 2+ (BSL2+)

In certain circumstances, experiments with BSL2 agents are approved only at BSL2+ or enhanced BSL2 containment. This means that work can be done in an ordinary BSL2 laboratory but BSL3 work practices are to be utilized.

In other circumstances, some existing facilities may not have features recommended for BSL3 (i.e. double-door access zone and sealed penetrations). An acceptable level of safety for the conduct of routine procedures (e.g. diagnostic procedures involving the propagation of an agent for identification, typing, susceptibility testing, etc.) may be achieved in a Biosafety Level 2 facility, provided that:

- exhaust air from the laboratory room is discharged to the outdoors;
- directional airflow into the laboratory is ensured;
- there is controlled access to the laboratory when work is in progress; *and*
- recommended BSL3 work practices are rigorously followed.

However, the implementation of such modification of Biosafety Level 3 should strictly be made upon recommendation of the IBC.

4.4.4 Biosafety Level 3 (BSL3)

Biosafety Level 3 (BSL3) is appropriate for agents with a known potential for **aerosol transmission**, for agents that may cause **serious and potentially lethal infections** and that are indigenous or exotic in origin.

Primary hazards to personnel working with these agents include autoinoculation, ingestion and exposure to infectious aerosols.

Greater emphasis is placed on primary and secondary barriers to protect personnel in adjoining areas, the community and the environment from exposure to infectious aerosols.

All laboratory manipulations are performed in a biological safety cabinet or other approved enclosed equipment; and personnel must wear appropriate personal protective clothing and equipment.

Secondary barriers include controlled access to the laboratory and a specialized ventilation system that minimizes the release of infectious aerosols from the laboratory.

Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

4.4.5 Biosafety Level 4 (BSL4)

BSL4 is applicable for work with dangerous and exotic agents which pose a **high individual risk of aerosol-transmitted laboratory infections and life-threatening disease with no treatment available**. Agents with close or identical antigenic relationship to Biosafety Level 4 agents should also be handled at this level.

All manipulations of potentially infected materials and isolates pose a high risk of exposure and infection to personnel, the community and the environment.

Primary hazards to personnel include respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets and autoinoculation.

Isolation of aerosolized infectious materials is accomplished primarily by working in a Class III biological safety cabinet or a full-body, or Class II biological safety cabinet with an air-supplied positive pressure personnel suit.

Access to the laboratory is strictly controlled.

The facility is generally a separate building or a completely isolated zone within a complex with specialized ventilation and waste management systems to prevent release of viable agents to the environment.

Laboratory personnel have specific and thorough training in handling extremely hazardous infectious agents and understand the primary and secondary containment functions of the standard and special practices, the containment equipment, and the laboratory design characteristics. They are supervised by competent scientists who are trained and experienced in working with these agents.

A summary of requirements pertaining to each of the biosafety levels (BSLs) for activities involving infectious materials is presented in the table below (adapted from WHO [Laboratory Biosafety Manual, 3rd Edition](#)).

Table 4.2 BSL requirements for working with agents of various risk groups

Risk Group	Biosafety Level	Laboratory Type	Laboratory Practices	Safety Equipment
1	BSL 1	Basic teaching, Research	General Microbiological Techniques	None; open bench work.
2	BSL 2	Primary health services; diagnostic services, research	General Microbiological Techniques plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosol.
3	BSL 3	Special diagnostic services, research	As level 2 plus special clothing, controlled access	BSC and/or other primary containment devices for all activities. Directional airflow, negative room pressure, exhaust air is HEPA-filtered.
4	BSL 4	Dangerous pathogen units	As level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall). Directional airflow, negative room pressure, supply air is HEPA-filtered, exhaust air is double-HEPA-filtered.

4.4.6 Risk Groups and BSL Handling Requirements

Generally, the direct relationship between the Risk Group of a micro-organism and the minimum level of containment under which it can be handled can be followed e.g. Containment Level 2 for Risk Group 2. However, depending on the characteristics of the micro-organism, the nature of the work and features of the exposed individuals, additional precautions may be required or exceptions can be made, sometimes on a case-by-case approach. Examples include:

- a. Handling requirements of Risk Group 1 agents may be adjusted to BSL2 for high concentrations, increased pathogenic potential or aerosolization during handling.
- b. Some Risk Group 3 agents (e.g. HIV, HTLV, SIV, other lentiviruses) where the risk of airborne transmission is low, can in some circumstances, be handled under less stringent conditions than BSL3 (i.e. BSL2+, which means BSL2 laboratory and safety equipment, and BSL3 procedures).
- c. BSL2+ containment rather than BSL2 should be considered for work involving co-cultivation / co-infection, virus replication studies, or manipulations involving concentrated virus or increased quantities of virus while virus production activities, including virus concentrations, require a BSL3 facility and use of BSL3 practices and procedures. For example, routine diagnostic work with BSL2 agents such as Hepatitis B and C Viruses, Human Immunodeficiency Virus (HIV) or lentiviruses can be done safely at BSL2 but may require a higher level of containment if large volumes or high risk procedures are used.
- d. **Bacterial Vectors**
Work with bacterial vectors containing Hepatitis B virus, Hepatitis C virus or lentivirus DNA (with no more than 60% of the viral genome), which are used to transform bacteria, can be done in a BSL2 laboratory with BSL2 procedures (appropriate DNA inactivation methods must be adopted). However, if the host cells produce replication-competent Risk Group 3 viruses, the appropriate safety level has to be considered (BSL2+ or BSL3).
- e. **Viral Vectors**
- Replication defective viral vectors that are not infective for mammalian cells and containing oncogene and/or cytokine-encoding sequences can be handled at BSL1. However, if the vectors consist of a heterologous envelope (e.g. VSV-G), handling at BSL2 may be required since the vectors have the potential to transduce human cells.
 - Replication-competent viral vectors that are infective for mammalian cells (excluding human and non-human primate cells) may be handled under BSL2.
 - Replication-defective viral vectors that are infective for human and non-human primate cells may be handled under BSL2.
 - Replication-competent vectors that are infective for human and non-human primate cells should be handled at a biosafety level according to the risk group of the wild-type virus.
- f. **Hazardous DNA Fragments**
- Experiments with naked lentiviral DNA including HIV require a BSL2 facility and procedures (appropriate DNA inactivation methods must be adopted).
 - Caution is required when working with nucleic acid sequences that encode proteins with gene-regulating functions (proteins with oncogenic potential, e.g. transcription factors, GTP-binding proteins, protein kinases, growth factors, etc.) or biologically active gene products (cytokines, growth hormones, toxins).
- g. **Fusion Proteins**
HIV-1 TAT and HSV VP22 fusion proteins can possibly have a negative effect on humans. While cloning and plasmid production can be safely carried out in a BSL1 setting, the expression of the proteins requires a BSL2 environment (appropriate protein inactivation methods must be adopted).

A summary of Risk Group (RG) and BSL handling requirements is presented in the table below as a general guide (adapted from [NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules \(NIH Guidelines\)](#)).

Table 4.3 Risk Groups and Biosafety Levels pertaining to various agents and lab activities

Risk Group	Classification	Examples of Agents, Tissues and Procedures in this Risk Group (RG)	Handling Requirements
1	Agents not associated with disease in healthy adult humans	Agents: -<i>Escherichia coli</i>-K12, asporogenic <i>Bacillus subtilis</i>, <i>Saccharomyces cerevisiae</i>, bacteriophages, low-risk oncogenic viruses (e.g. SV 40, mouse mammary tumor virus), adeno-associated virus types 1 through 4, - Genetic sequences encoding for gene-regulating proteins (oncogenes etc.) in replication-defective bacterial vectors. Tissues: Tissues from animals infected with RG1 agents. Procedures: -Most routine laboratory procedures involving well-characterized agents not known to cause disease in man. -Cloning and production of fusion proteins.	Generally, Biosafety Level 1 (BSL1) but may be adjusted to BSL2 for high concentrations, increased pathogenic potential, or aerosolization during handling.
2	Agents associated with human disease which is rarely serious and for which preventive or therapeutic interventions are <i>often</i> available	Agents: -<i>Salmonella</i> and <i>Legionella</i> species, enteropathogenic <i>E. coli</i>, <i>Cryptococcus neoformans</i>, adenoviruses (including replication-defective strains), hepatitis viruses, all polioviruses, rabies virus; amphotropic and xenotropic murine and avian retroviruses, vectors containing full length lentiviral (including HIV) material. -Genetic sequences encoding for gene-regulating proteins (oncogenes etc.) in replication-defective vectors for human and non-human primate cells, replication-competent vectors for mammalian cells (excluding human and non-human primate cells). Tissues: Human or other primate blood, body fluids and tissues; cell cultures of human origin. Procedures: -Minimum level for Category A and B recombinant DNA experiments as defined by GMAC; e.g. introducing recombinant DNA (rDNA) into RG2 agents, or DNA from RG2 or RG3 agent into nonpathogenic prokaryote or lower eukaryote; working with replication-defective RG2 virus in presence or absence of helper virus. -Cloning and production of fusion proteins.	Generally, Biosafety Level 2 (BSL2) but may be adjusted up or down depending upon specific conditions of use.

Risk Group	Classification	Examples of Agents, Tissues and Procedures in this Risk Group (RG)	Handling Requirements
3	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)	Agents: -<i>Myobacterium tuberculosis</i>, <i>Coxiella burnetii</i>, <i>Yersinia pestis</i>, <i>Histoplasma capsulatum</i>, prions, HIV, HTLV, and most arboviruses. -Genetic sequences encoding for gene-regulating proteins (oncogenes etc.) in replication-competent vectors for human and non-human primate mammalian cells. Tissues: Placental tissues from sheep infected with <i>C. burnetii</i>. Procedure: Introducing rDNA into RG3 agents.	Generally, Biosafety Level 3 (BSL3) but may be adjusted up or down depending upon specific conditions of use.
4	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are <i>not usually</i> available (high individual risk and high community risk)	Agents: Only viruses, including Lassa, Junin, Machupo, Ebola and Marburg viruses and <i>Herpesvirus simiae</i> Tissues: Specimen from any individual infected with any RG4 agent. Procedure: Introducing DNA from a RG4 agent into a non-pathogenic prokaryote or lower eukaryote <u>without</u> demonstrating that a totally and irreversibly defective fraction of the agent's genome is present in the recombinant.	Biosafety Level 4 (BSL4).

Further details can be found in:

1. WHO "[Laboratory Biosafety Manual, 3rd Edition.](#)"
2. GMAC guidelines, "[Singapore Biosafety Guidelines for Research on Genetically Modified Organisms \(GMOs\)](#)".

4.5 VERTEBRATE ANIMAL BIOSAFETY LEVELS

There are four animal biosafety levels, designated Animal Biosafety Level (ABSL) 1, ABSL2, ABSL3, and ABSL4, for work with infectious agents in animals. The levels are combinations of work practices, safety equipment and facilities for experiments on animals infected with agents which produce or may produce human infection. In general, the biosafety level recommended for working with an infectious agent *in vivo* and *in vitro* is comparable.

Only ABSL1 and ABSL2 facilities are operated by the university.

Animal Biosafety Level 1 (ABSL-1) is suitable for work involving little or no known potential hazard to animal handling personnel and the environment.

Animal Biosafety Level 2 (ABSL2) is suitable for animal work involving inoculation of agents of moderate potential hazard to personnel and the environment. Under NUS requirement, it is also applicable to work with animals inoculated with materials of human origin (human cell lines, tissues, body fluids, etc.) from both commercial and non-commercial sources.

Animal Biosafety Level 3 (ABSL3) is suitable for work involving BSL3 agents may cause serious or lethal disease by the inhalation of aerosols or dust particles or the parenteral route (sharps, biting, scratching).

Animal Biosafety Level 4 (ABSL4) is required for all work with dangerous and exotic agents which pose a high individual risk of aerosol transmitted laboratory infections and life threatening disease.

The practices, safety equipment, and facilities requirements for the different biosafety levels are specified in the [NUS Occupational Health and Safety Manual for Personnel with Research Animal Contact](#).

4.6 GUIDELINES FOR DETERMINING BIOSAFETY LEVEL

The appropriate Biosafety Level (BSL) must be assigned for each research project and the associated biological agent(s). Good references for assigning BSLs can be obtained from:

1. [Laboratory Biosafety Manual, 3rd Edition](#), World Health Organization, 2004.
2. [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\), 5th edition](#), National Institutes of Health, Department of Health and Human Services, USA, 2007 - Section II on risk assessment.
3. [Pathogen Safety Data Sheets and Risk Assessment](#), Public Health Agency of Canada - Material Safety Data Sheet (MSDS) section.
4. *Laboratory-acquired infections*, ed. C.H. Collins, Butterworth Publications, London, 2nd Edition, 1988.
5. [Biological agents: Managing the risks in laboratories and healthcare premises](#), Advisory Committee on Dangerous Pathogens, Department of Health, United Kingdom, 2003.

The following flowchart shows how the biosafety level can be determined for the most widely used biological agents and toxins. If animals are involved in the research, the corresponding animal biosafety levels apply.

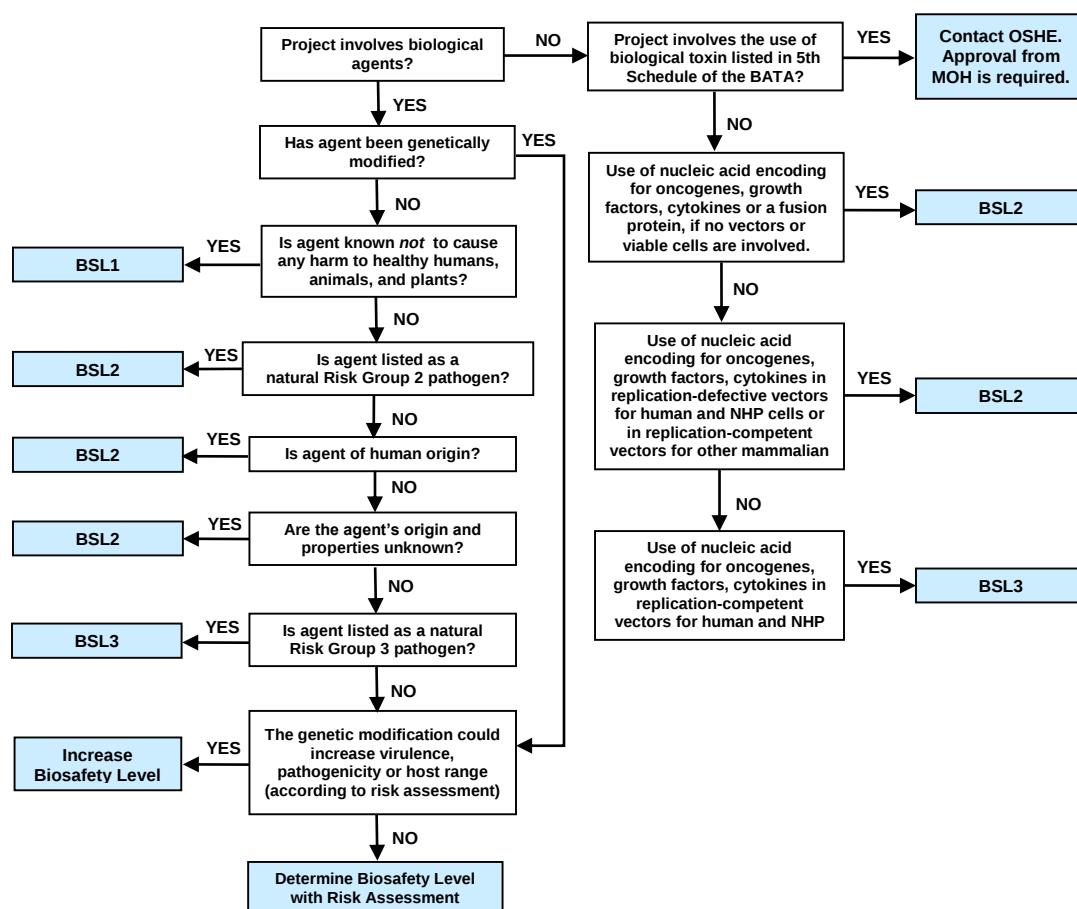


Figure 4.1 Flowchart on determination of biosafety levels

4.6.1 Risk group is known. First, identify the risk group of the biological agent (if available) and determine the BSL or ABSL assigned for the biological agent as shown in the flowcharts above. The [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\), 5th edition](#) publication and the Public Health Agency of Canada's [Pathogen Safety Data Sheets and Risk Assessment](#) section are useful references which provides agent summary statements for some agents and information on the associated hazards and recommended precautions.

4.6.2 Well-characterized harmless agents, knowingly not causing any harm to humans, animals and plants can be used in a BSL1 setting.

4.6.3 If no viable cells are used. For work with biological toxins, certain nucleic acids and their products, it makes a difference if these substances are used in vectors or viable cells as shown in the flowchart above. The risk assessment has to provide the necessary information for the decision making. Some biological toxins fall under the Fifth Schedule of the BATA and the facility, the administrative controls and PPE must strictly meet the BATA requirements. Please contact OSHE as approval must be sought from MOH. Please refer to the website for the current [List of Biological Agents and Toxins](#) under the BATA.

4.6.4 Activities with increased risks. The BSLs assigned to a particular agent assumes activities typically associated with the routine growth and manipulations of infectious agents at quantities and concentrations to accomplish identification or typing. However, if the activity involves higher agent concentrations, larger agent volumes, or practices likely to endanger personnel or the environment (public, wildlife, plants, habitats), additional precautions may be required and the BSL assignment may have to be increased.

A risk assessment strategy that may be used to determine if changes in an assigned BSL are required is shown below. At each stage in the assessment, a subjective evaluation considering the infective potential of the agent involved and the gravity of the infection must be made.

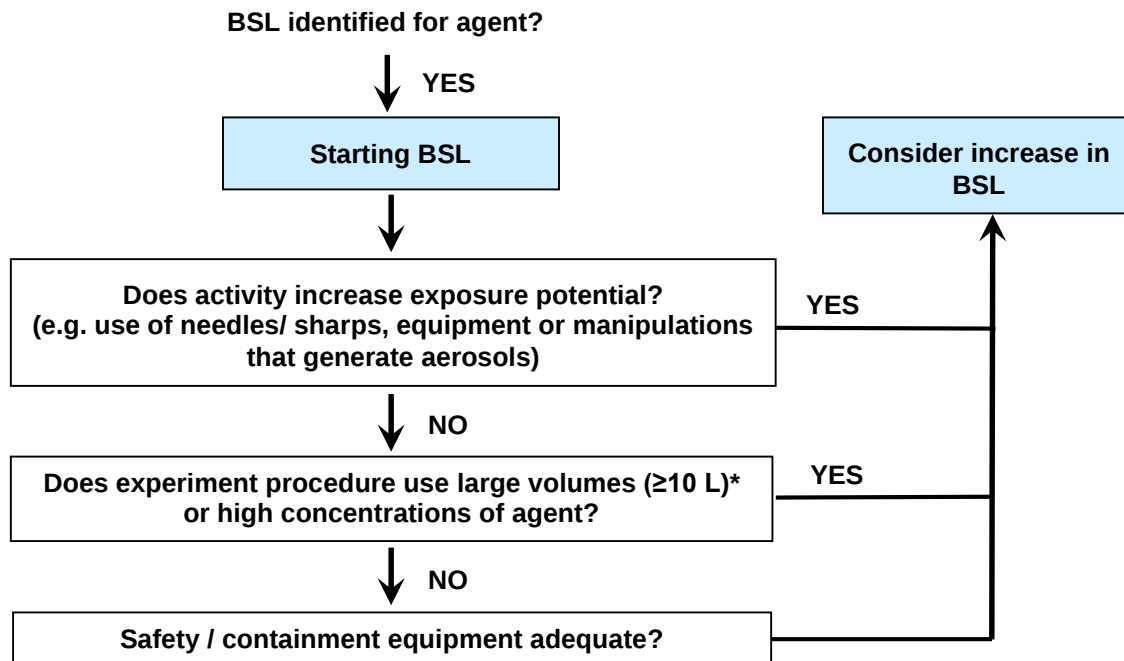


Figure 4.2 Flowchart on Risk Assessment strategy

* Note: For large-scale experiments, an application with MOH is necessary for certain agents listed under Schedule 3 of the Biological Agents and Toxins Act.

4.6.5 Risk group is not known. If the BSL assignments of a biological agent is not known or there is insufficient information e.g. unknown agent that may be present in a diagnostic specimen, make a preliminary determination of the biosafety level that best correlates with the initial risk assessment based on the identification and evaluation of the agent hazards. It would be prudent to assume the specimen contains an agent presenting the hazardous classification that correlates with BSL2 unless additional information suggests the presence of an agent of higher risk. Consider routes of agent transmission, means of transmission and the infective potential of the agent. The strategy shown below could be adopted. At each stage, assess the best available information to make an evaluation.

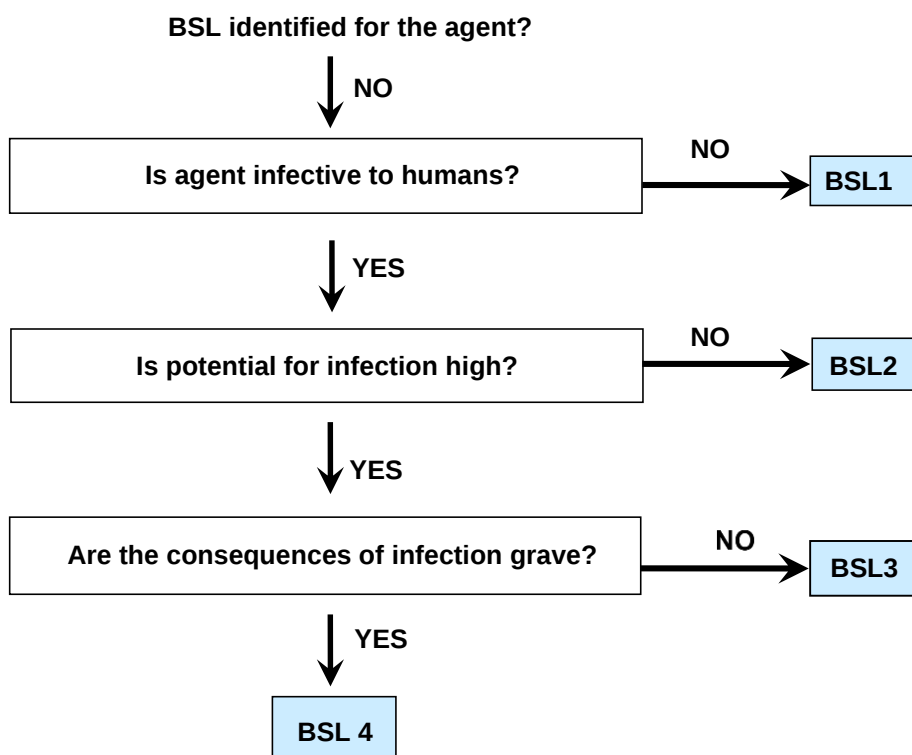


Figure 4.3 Flowchart on Biosafety Level strategy

4.6.6 Genetically modified agents. If experiments involving genetic manipulation or recombinant DNA are to be carried out, BSL assignment follows the same guidelines as the wild-type biological agent with additional considerations of possible risk associated with changes in the agent's pathogenicity or susceptibility to current treatments as a result of the modification. For experiments involving recombinant viral vectors, BSL assignment is dependent on the ability of the agent to infect mammals and/or humans. For experiments involving certain hazardous nucleic acid material or proteins, the risk is assessed in a similar way; consider the health-risk for humans, routes and means of infections and the infective potential of the agent.

First, determine the applicable biosafety level for the agent without the genetic modification. If the modification could increase the agent's virulence, pathogenicity or host range (according to the risk assessment), the biosafety level has to be increased. If the genetic modification has been proven to lower the agent's risk, and this has been generally accepted, the biosafety level can be decreased (e.g. as for *Escherichia coli* K12).

The following sources of information are useful references that can be used to assist in assessing risk and establishing appropriate biosafety level for work involving recombinant DNA molecules:

- [Singapore Biosafety Guidelines for Research on Genetically Modified Organisms \(GMOs\)](#), 2013.
- [NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules \(NIH Guidelines\)](#), National Institutes of Health, Department of Health and Human Services, USA, 2002.

4.6.7 The final selection of the appropriate biosafety level and the selection of any additional laboratory precautions must take into consideration all pathogen, host and environmental factors in a risk assessment. This requires a comprehensive understanding of the practices, safety equipment, and facility safeguards (refer Chapters 5-12).

4.7 SAFETY GUIDELINES FOR BENCH-WORK IN SHARED LABORATORIES

There is an increasing trend of research laboratories being designed in an “open” concept, instead of individual laboratory suites. This layout provides greater opportunities for collaboration and for monitoring of safety practices. As there might be different research groups in these laboratories, there is a need for each group to communicate their hazards and risks to the other research groups.

4.7.1 Scope

The guidelines are applicable to all NUS students, staff, collaborators and visitors who perform laboratory based research activities in “open” benches in shared laboratories.

4.7.2 Safety Guidelines

- a. The PI shall conduct a risk assessment of the activities and determine if the activities can be conducted in the open bench.
- b. The PI shall ensure that the controls for ensuring the health and safety of the other researchers in the laboratory are in place. This should include:
 - Relevant hazard warning signs shall be posted to communicate the hazards to other occupiers of the shared laboratory. For example, if research activity involving animals are performed in a shared lab, a warning sign stating “Animals may be used in labs” shall be posted on the entrance to the laboratory door.
 - The PI shall identify possible emergency situations in the shared laboratory that may arise from the research activities conducted by their respective group. PI shall also ensure appropriate emergency response procedures are developed for such situations and these are communicated to other occupiers in the shared laboratory.
 - Determining if researchers in the neighboring benches require specific risk controls such as Occupational Health monitoring or specialized Personal Protective Equipment. This should be determined based on the level and duration of exposure of the hazard to the researchers working in neighbouring benches.

4.8 RISK ASSESSMENT REVIEW

All staff undertaking new lab-based research projects (PIs who are not certified under Laboratory OHS Certification Scheme) must carry out a project-based risk assessment and submit it to the IBC for review. The risk assessment can be submitted to OSHE via the [integrated Online Research Compliance \(iORC\)](#).

OSHE will conduct a preliminary review of the risk assessment on behalf of the IBC. Higher risk projects or projects whereby OSHE does not have the expertise or competency to evaluate the risk assessments will be reviewed by the IBC or government agencies.

PIs awarded certification under the [Laboratory Safety & Health Management System Certification Scheme](#), shall conduct ongoing hazards identification, risks assessment (activity/experiment-based), and determination of necessary controls for all the laboratory activities, including both day-to-day activities and those conducted only periodically or on an ad-hoc basis and document under the OH & S management system dossier, [NUS Occupational health and safety \(OH&S\) management system standard for laboratories - Part A: Requirements](#).

The progress in modern genomics and molecular biology entails that not all biological hazards of activities with viruses, vectors and nucleic sequences as well as gene products can be covered in this manual and appropriately grouped and classified in advance. It falls under the PI's responsibilities and duties to identify potential hazards and inform the Head of Department and the IBC about planned activities that are not clearly regulated. In preparation for an evaluation and decision by the IBC, the PI has to prepare the risk assessment documentation, which includes scientific safety-related literature and/or existing recommendations discussed within the scientific community.

CHAPTER 5 ENGINEERING CONTROLS

Engineering controls are tools or equipment that provide protection to the operator and the environment when used correctly. They consist of various measures for reducing a hazard at its source through containment or for separating personnel from the hazard. Examples include laminar flow hoods, biological safety cabinets, glove boxes, chemical fume hoods, vacuum line chemical traps and filters, to name a few. Correct use of these pieces of equipment is critical. Engineering controls do not eliminate hazards, but rather isolate people from hazards. Capital costs of engineering controls tend to be higher than those less effective controls within the hierarchy, however they may reduce future costs. Engineering controls should be used in conjunction with personal protection and administrative controls (see hierarchy of controls, Chapter 4, Risk Management).

5.1 LAMINAR FLOW HOODS / CLEAN BENCHES

Laminar flow hoods or clean benches discharge a laminar flow of filtered air from top to bottom or across the work surface and toward the user, providing *product protection only* and must not be used when working with any form of biohazard or chemical hazard. In both horizontal and vertical-flow laminar hoods, air is pushed through a High Efficiency Particulate Air (HEPA) or Ultra-Low Penetration Air (ULPA) filter, and out across the bench towards the operator and into the laboratory space. Air from the room is kept from blowing into the hood so air flowing across the workspace is free from particulates. These units provide *product protection only* by ensuring that the product is exposed only to filtered air. Any infectious or allergenic aerosols, chemical fumes, or vapors generated will be blown out toward the user and into the laboratory space. Hence, these units do not provide protection to personnel or the ambient environment, and are **not designed for use with infectious, chemical, or radiological materials**.

This equipment is suitable for procedures such as pouring of agar plates or processing non-hazardous material, and for certain clean activities, such as dust-free assembly of sterile equipment or electronic devices. However, they should never be used when handling cell culture materials or potentially infectious materials, or as a substitute for a biological safety cabinet in research laboratories.

Compared with horizontal flow models, vertical flow clean benches generate less turbulence when large instruments or items are placed in the work zone.

HEPA/ULPA filters provide a range of high performance protection. These self-contained filters are designed to physically capture particles larger than 0.3 microns (HEPA) or 0.12 microns (ULPA) with >99.999% typical efficiency.

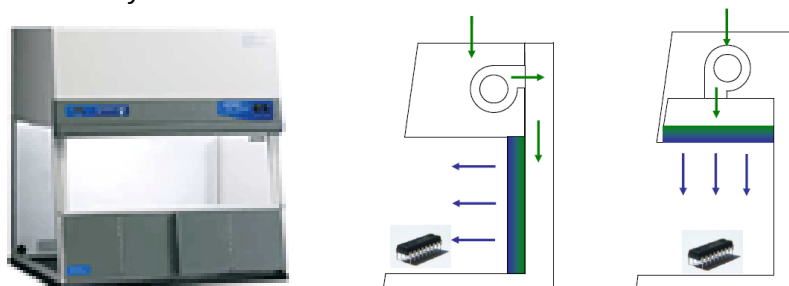


Figure 5.1 Example of a laminar flow hood, and horizontal and vertical flow models

5.2 BIOLOGICAL SAFETY CABINETS

Biological safety cabinets (BSCs) represent one of the most important biosafety engineering controls. They should not be mistaken for laminar flow hoods or chemical fume hoods. They are enclosed, ventilated work spaces for safely working with materials contaminated (or potentially contaminated) with pathogens. BSCs are designed to provide *personnel, environmental and product protection* when good microbiological practices are followed while working in them, except for Class I cabinets which provide personnel and environment protection only. BSCs provide this protection through the use of laminar air flow and HEPA filtration. Three types of BSCs (Class I, II and III) are used in laboratories. The partially open-fronted Class II BSCs are the most common containment devices used in research laboratories and offer significant levels of protection to laboratory personnel and to the environment when used in combination with good laboratory technique.

Biological safety cabinets must be certified annually by a qualified person.

5.2.1 Definitions

Biological Safety Cabinet (BSC): Air flow controlled hoods designed to protect the operator, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating materials containing infectious agents, such as primary cultures, stocks and diagnostic specimens.

High Efficiency Particulate Air (HEPA): Filter that traps 99.97% of particles of 0.3 microns in diameter, thus, capturing all infectious agents and ensuring only microbe free air is exhausted from the cabinet.

Class I cabinets protect personnel and the environment, but not research materials. They provide an inward flow of unfiltered air, similar to a chemical fume hood, which protects the worker from the material in the cabinet. The environment is protected by HEPA filtration of the exhaust air before it is discharged into the laboratory or to the outside via the building exhaust.

Class II cabinets (Types A1, A2, B1, B2) provide personnel, environment, and product protection. Air is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air within the cabinet provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air passes through the exhaust HEPA filter, it is contaminant-free (environment protection), and may be circulated back into the laboratory (Type A) or ducted out of the building (Type B). The Class II BSC can be used for working with infectious agents in Risk Groups 2 and 3. Class II BSCs can be used for working with infectious agents in Risk Group 4 when positive-pressure suits are used.

Class III cabinets (sometimes called Class III glove boxes) were designed for work with infectious agents in Risk Group 4, and provide maximum protection to the environment and the worker. The cabinet is gas-tight with a non-opening view window, and has rubber gloves attached to ports in the cabinet that allow for manipulation of materials in the cabinet. Air is filtered through one HEPA filter as it enters the cabinet, and through 2 HEPA filters before it is exhausted to the outdoors. There may also be requirements regarding the connection to a dedicated exhaust system. This type of cabinet provides the highest level of product, environmental, and personnel protection. Class III BSCs are suitable for work in Biosafety Level 3 and 4 laboratories.

The choice of BSC should be one of the controls included in a risk assessment for working with

infectious agents, potentially infectious biological materials, etc.

5.2.2 Preparing to Work in a Class II BSC

- a. Prepare a written checklist of materials required for the activities to be performed in the BSC.
- b. Gather all materials needed before starting procedures to minimize stopping to retrieve supplies, etc. and bringing the arms out of the BSC once work has commenced.
- c. Volatile or toxic chemicals should not be used in Class II A1 or Class II A2 BSCs that recirculate exhaust air to the room (these are the most common types of BSC found in NUS research laboratories).
- d. Turn on the fan for at least 10 minutes.
- e. Disinfect the surfaces inside the BSC and of all items which will be placed in the BSC with an efficacious chemical disinfectant (see Chapter 8 on choosing an appropriate disinfectant).
- f. Arrange the materials inside the BSC from “clean to dirty” to minimize any possible cross-contamination and so that the work proceeds from the clean side to the dirty side containing the waste receptacles, etc.
- g. Plastic-backed absorbent toweling can be placed on the work surface but not on the front or rear grille openings. The use of toweling facilitates routine cleanup and reduces splatter and aerosol generation during an overt spill. It can be folded and placed in a biohazard bag or other appropriate receptacle when work is completed.
- h. All materials should be placed as far back in the cabinet, towards the rear edge of the work surface, as practical without blocking the rear grille. Aerosol-generating equipment (e.g. mixers, centrifuges, etc.) should be placed towards the rear of the cabinet.
- i. Waste collection bags, trays, etc. should be placed inside the BSC in the “dirty” side to minimize the movement of the arms in and out of the fragile “air barrier” in the opening of the BSC. Such frequent movement can compromise both the personnel and product protection.
- j. Pipette collection containers should be flat, horizontal trays or boxes (not upright canisters or beakers which can fall over).
- k. Vacuum waste collection flasks can be placed below the BSC if placed in a leak-proof secondary container (i.e. plastic pan) and disinfectant (enough to be active in the volume of waste to be collected) is placed in the flasks before waste is collected and disinfectant is also added at the end of the procedures (see Chapter 8, Decontamination).
- l. A hydrophobic vacuum line filter meeting HEPA specifications (filtering at least 99.97% of particles greater than 0.3 μm) should also be placed in the vacuum line between the vacuum and the last flask to protect the vacuum and the environment from any aerosols which may be created in the vacuum flask during the process of waste collection (see Section 5.5 Vacuum Line Chemical Traps and Filters).
- m. The correct sash position (usually 20-25 cm) above the base of the opening should be indicated on the front of the cabinet. On newer BSCs, an audible alarm will sound if the sash is in the wrong position while the fan is operating.
- n. Ensure that the chair or stool is at the correct ergonomic height and that the face is above the front opening.
- o. Laboratory coats should be worn buttoned over street clothing.
- p. Latex, vinyl, nitrile or other suitable gloves are worn to provide hand protection.
- q. Increasing levels of PPE may be warranted as determined by an individual risk assessment. For example, a solid front, back-closing laboratory gown provides better protection of personal clothing than a front-buttoned laboratory coat and is a recommended practice at BSL-2+ (BSL2 enhanced) or BSL3.

5.2.3 Operation

- a. Gloves should overlap the sleeves of the laboratory coat or gown. This protects the workers skin from splashes as well as protects the “products” in the BSC from any particles shed by the worker’s skin.
- b. Arms should be moved in and out slowly, perpendicular to the front opening to minimize disruption of the air curtain and laminar flow. Manipulations of materials within BSCs should be delayed for about 1 minute after placing hands and arms inside to allow the cabinet to adjust and to “air sweep” the surface of the hands and arms.
- c. Work as far to the back (beyond the air split) of the BSC workspace as possible, but within comfortable reach.
- d. Always use mechanical pipetting aids. *No mouth pipetting is allowed.*
- e. Heat sources such as Bunsen burners are strictly prohibited inside the BSCs as they significantly disrupt the laminar flow of air.
- f. Sterilization of bacteriological loops using microburners or electric “furnaces” is NOT recommended to be done inside the BSC. This procedure can be done at an open workbench, with a stainless steel shield enclosing the burner to prevent the flame from spreading.
- g. Do not work in a BSC while a warning light or alarm is signaling.

5.2.4 Ventilation Rates and Negative Pressure Requirements

All Class I and II BSC face velocities shall be sufficient to maintain an inward flow of air at all openings into the cabinet under operating conditions. Velocity measurements shall be made at the work opening of the cabinet with a calibrated anemometer. The ability of the biological safety cabinet to maintain an inward flow shall be demonstrated using smoke tubes or other suitable qualitative methods

- a. The mechanical ventilation system in a Class I and Class II (Type A1) BSC shall provide a minimum inward average face velocity of 0.38 m/s (75 linear feet per minute) at the work opening.
- b. The mechanical ventilation system in a Class II (Type A2, B1, B2) BSC shall provide a minimum inward average face velocity of at least 0.51 m/s (100 linear feet per minute) at the work opening
- c. The mechanical ventilation system in a Class III biological safety cabinet shall provide sufficient air flow to maintain a constant purging of the work area of hazardous vapors, gases or particulate generated within the cabinet and to dilute flammable dusts, gases, or vapors to below 20% of the lower explosive limit (LEL) at a minimum negative pressure inside the cabinet of 0.5 inches of water gauge. The airflow through the Class III biological safety cabinet shall be determined by measuring the exhaust velocity at the exhaust port. Total air volume is calculated by the following equation: (exhaust velocity) X (area of exhaust port) = total air volume. The air change rate for a class III biological safety cabinet shall be a minimum of 1 air change in 3 minutes or airflow required to maintain flammable gases/vapors below 20% of the LEL whichever is greater. The measurement of the negative pressure inside the cabinet shall be made with a calibrated gauge. The accuracy of the gauge shall be +5% at the required 0.5 inches of water gauge.

5.2.5 Spillage

- a. If there is a spill of biological material in the BSC, absorb the liquid and place contaminated absorbent material in a biohazard bag in the BSC; surface decontaminate all objects in the cabinet with proper disinfectant; disinfect the working area of the cabinet while the fan is still in operation (do not turn the cabinet off) (refer to Chapter 12, Section 12.3 Spill Response).
- b. If spillage occurs outside the cabinet, inform your supervisor immediately to activate your spill control/response group, if necessary, depending upon the size of the spill.

5.2.6 Cleaning and Disinfection

- a. When work is completed, all equipment and supplies from the cabinet should be surface-decontaminated and removed from the cabinet.
- b. The interior surfaces should also be wiped with an appropriate disinfectant that would kill any microorganisms that could be found in the cabinet. Corrosive chemicals such as sodium hypochlorite can be used, but should be followed with a second wipe down with sterile water.
- c. Allow the cabinet to run for 5 minutes before switching off.

5.2.7 Decontamination

Space decontamination (usually gaseous or vapor decontamination) of the air plenums and mechanical areas of the BSC is mandatory when maintenance work, filter changes, and performance tests require access to any contaminated portion of the cabinet. All work surfaces and exposed surfaces should be decontaminated with a suitable surface disinfectant before certification tests are performed and before gaseous decontamination takes place. In addition, it may be desirable to perform gaseous decontamination of the entire cabinet before performing certification tests when the cabinet has been used with agents assigned to biosafety level 2, and is recommended when the cabinet has been used with an agent assigned to biosafety level 3. A qualified safety and risk assessment of cabinets potentially contaminated with biological agents should be performed by a biosafety officer or qualified safety professional.

- a. Appropriate decontamination (space and/or surface) should be performed before BSCs are moved to another location. Additionally, after spills and splashes of infectious agents, contaminated surfaces should be appropriately decontaminated. International certification standards require that BSCs must be decontaminated before filter changes, as the contaminated plenum must be accessed. Depending upon the agents worked with in the BSC and upon the risk assessment, the BSC may also need to be decontaminated before being moved.
- b. BSC decontamination should be performed by a qualified professional. The most common decontamination method uses fumigation with formaldehyde gas while the BSC is sealed in plastic sheeting. Since formaldehyde is an irritant and suspected of being carcinogenic for humans, students/staff are discouraged from coming in contact with the BSC while the decontamination process is being performed.
- c. Vaporized hydrogen peroxide (VHP) and chlorine dioxide have also been recently validated as methods for decontaminating BSCs.

5.2.8 UV Lights

Some BSCs are equipped with ultraviolet (UV) lights. Germicidal (or UV) lamps were often installed in the past as an adjunct to surface disinfection. However, currently UV lighting is not recommended in BSCs by the BSC manufacturers, American Biological Safety Association, or other international associations. The NSF 49 2010 Standard for certifying BSCs does not provide any performance verification of UV lighting.

UV irradiation can cause erythema of skin and eye damage. Therefore, the UV lamp should never be “on” while an operator is working in the cabinet. Proper shielding (i.e. the sash is closed) must be in place when the UV light is turned on.

Only the 245 nm wavelength is considered to have biocidal effects. However, a much broader spectrum will give “blue light” and special equipment must be used to verify that the biocidal wavelength is being emitted. Even if the 245nm wavelength is present, it will have no effect in areas where supplies or

equipment are stored or cast shadows in the cabinet. UV light does not penetrate surfaces.

UV radiation should not take the place of thorough chemical disinfection of the surface of the cabinet interior. That is why it is so important to choose a disinfectant that is efficacious for the organisms which may be present in the materials handled in the BSC (see Chapter 8.2 Selecting Chemical Disinfectants).

While their usefulness is a subject for debate between some users and manufacturers, purchasers can request (and pay extra) to have UV lamps installed in the BSC. However, they must be installed by the manufacturer and in such a manner that it does not reduce the required performance of the other criteria of the BSC. UV lamps should not be retrofitted into existing BSCs by maintenance personnel.

5.2.9 Field Testing

Each cabinet should be field tested at the time of installation and at least annually thereafter. In addition, recertification should be performed whenever HEPA/ULPA filters are changed, maintenance repairs are made to internal parts, or a cabinet is relocated. More frequent recertification should be considered for particularly hazardous or critical applications or workloads.

At NUS, the person conducting the designated tests must affix to the cabinet a certificate of satisfactory performance when the cabinet meets all field test criteria.

The following physical tests shall be conducted on-site for a certification to qualify for the statement "Field Certified in accordance with NSF/ANSI 49":

- downflow velocity profile test
- Inflow velocity test
- airflow smoke patterns test
- HEPA/ULPA filter leak test
- cabinet integrity test (positive pressure plenum cabinets only)
- site installation assessment tests

The site installation assessment tests shall include:

- alarm functions as required by NSF/ANSI 49
- blower interlock
- exhaust system performance (proper exhaust duct negative pressure and canopy performance, if applicable)

The following tests are for worker comfort and safety and are performed at the request of the customer or at the discretion of the certification provider:

- lighting intensity
- vibration
- noise level
- electrical leakage, ground circuit resistance, and polarity tests

5.2.9.1 Downflow Velocity Test: This test measures the velocity of air moving through the cabinet workspace 4 in (10 cm) above the bottom edge of the window and shall be performed on all cabinets.

- a. the average downflow velocity shall be within ± 5 ft/min (± 0.025 m/s) of the value specified
- b. the individual point readings shall not vary more than $\pm 25\%$ or 16 ft/min (0.08 m/s) whichever is greater, from the average downflow velocity.

- 5.2.9.2 **Inflow Velocity Test:** This test determines the measured and calculated inflow velocity through the work access opening. The average work access opening inflow velocity shall be within ± 5 ft/min (± 0.025 m/s) of the nominal set point.
- 5.2.9.3 **Airflow Smoke Patterns Test:** This test determines that the airflow along the entire perimeter of the work access opening is inward, that airflow within the work area is downward with no dead spots or refluxing, that ambient air does not pass on or over the work surface, and that there is no escape to the outside of the cabinet at the sides and top of the window.
- a. Downflow test: The smoke shall show smooth downward flow with no dead spots or reflux (upward flow).
 - b. View screen retention test: The smoke shall show smooth downward flow with no dead spots or reflux. No smoke shall escape from the cabinet.
 - c. Work opening edge retention test: No smoke shall be refluxed out of the cabinet once drawn in, nor shall smoke billow over the work surface or penetrate onto it.
 - d. Sash/window seal test: There shall be no escape of smoke from the cabinet.
- 5.2.9.4 **HEPA/ULPA Filter Leak Test:** This test determines the integrity of downflow and exhaust HEPA/ULPA filters, filter housings, and filter mounting frames. The cabinet shall be operated within ± 5 ft/min (0.025 m/s) of the nominal set point.
- a. Filters that can be scanned: Sustained aerosol penetration shall not exceed 0.01% of the upstream concentration at any point.
 - b. Filters that cannot be scanned: Sustained aerosol penetration shall not exceed 0.005% of the upstream concentration.
- 5.2.9.5 **Site Installation Assessment Tests:** These tests are performed to verify that the biosafety cabinet is integrated properly into the facility.
- 5.2.9.6 **Lighting Intensity Test:** This test is performed to measure the light intensity on the work surface of the cabinet in foot-candles (lux) as an aid in minimizing cabinet operator's fatigue. Lighting intensities shall average no less than 45 ft-candles (480 lux) greater than background levels, where background light levels average a maximum of 15 ft-candles (160 lux).
- 5.2.9.7 **Vibration Test:** This test is performed to determine the amount of vibration in an operating cabinet as a guide to satisfactory mechanical performance, as an aid in minimizing cabinet operator's fatigue, and to prevent damage to delicate tissue culture specimens. Net displacement shall not exceed 0.002 in (50 μ m) rms amplitude at 10 Hz to 10 kHz in the center of the work surface(s) when the cabinet is operating at the manufacturer's recommended airflow velocities.
- 5.2.9.8 **Noise Level Tests:** This test is performed to measure the noise levels produced by the cabinet as a guide to satisfactory mechanical performance and an aid in minimizing cabinet operator's fatigue. The procedures can be performed in most acoustically ordinary rooms, such as a factory, where walls are neither sound absorbing nor completely sound reflecting.

Overall noise level in front of the cabinet shall not exceed 70 dBA when measured where the maximum ambient sound level is no greater than 60 dBA. Standard correction curves or tables shall be used (see below).

Table 5.1 Correction chart for sound level readings

Difference between total and background sound readings (dBA)	Number to subtract from total to yield correct noise level
0 - 2	Reduce background levels
3	3
4 - 5	2
6 - 10	1
>10	0

5.2.10 Frequency of Testing

The biological safety cabinet certification shall be performed under any of these following conditions:

- Upon installation
- At least once every 365 days
- When relocated from one place to another
- When changes have been made to the layout of the lab with the biological safety cabinet (addition / removal of equipment, infrastructure changes such as HVAC, etc.)

5.2.11 Other Considerations

- a. All test or maintenance activities requiring access to potentially contaminated interior spaces of the cabinet shall be performed after appropriate decontamination using methods approved in the NSF 49 standard.
- b. A warning placard shall be placed on the front of the cabinet requiring decontamination prior to opening any service panel or other interior access.
- c. Where biological safety cabinets are attached to external duct systems with a blower and the cabinet system also contains a blower, or where the cabinet uses an external blower, an audible and visual alarm system to alert the user indicating the loss of exhaust flow in the external duct shall be used.
- d. All repairs to the BSC should be done by a qualified technician. Any malfunction in the operation of the BSC should be reported and repaired before the BSC is used again.
- e. The BSC should be positioned in an isolated corner to minimize disruption of the air intake arising from traffic around the biosafety cabinet or drafts from doors and air conditioning. There should be at least a 30 cm clearance provided behind and on each side of the cabinet to allow easy access for maintenance. A clearance of 30 to 35 cm above the cabinet may be required to provide for accurate air velocity measurement across the exhaust filter and for exhaust filter changes.
- f. BSC must meet the requirements of international certification bodies such as the Canadian Standards Association (CSA) Z316.3-95, the National Sanitation Foundation (NSF, USA), or AS/NZ, etc.) and subjected to a yearly certification by a competent/qualified professional to an international standard. Re-certification is required each time the BSC is repaired or relocated.
- g. For BSC II Type B, an alarm that is audible at the cabinet should be provided to indicate loss of exhaust flow from the building exhaust system. The cabinet should also be interlocked with the building exhaust system to prevent pressurization of the cabinet in the event of building exhaust fan failure.

5.2.12 Certification Label Recordkeeping

Biosafety cabinets field tested shall include the following information on the label:

- date of certification
- date cabinet should be recertified: no later than
- certifier's report number (reference document showing tests performed and results)
- name, address, and telephone number of certifying company
- signature of the person who performed the field certification tests

A copy of the certification report must be kept in a file by the BSC owner for verification purposes. The report must have results and relevant information that were noted during the biological safety cabinet velocity testing, HEPA filter testing, airflow smoke testing and general safety testing. The report must also have the information about the detectors used and their calibration dates. The biological safety cabinet certification report must be submitted to the equipment owner within two weeks of the completion of the biological safety cabinet certification. The records must be retained by the equipment owner for three years from the certification date.

A certification report that will carry the language "certified in accordance with NSF" or similar language shall, at a minimum, include the following:

- a. BSC model number
- b. BSC serial number
- c. BSC location
- d. BSC venting information (ducted or not ducted; type of connection (canopy, direct or none))
- e. Type of BSC
- f. Test equipment used for each test:
- g. manufacturer
- h. model
- i. serial number
- j. calibration date
- k. Specific test data
- l. Acceptance criteria for each test
- m. Printed name of certification technician
- n. Test date
- o. Retest date

5.2.13 How to Choose a Vendor

- a. Vendors of BSCs must provide documentation of their competence to provide biological safety cabinet certification service to the equipment owner. Vendors should have a valid certification from a relevant authority to perform biological safety cabinet certification.
- b. Vendors must perform the biological safety cabinet certification according to the requirements of the specification.
- c. Vendors must ensure the detectors used for the certification are calibrated according to manufacturer requirements.
- d. Vendors must inform the equipment owner immediately of a biological safety cabinet failing certification and post a sign on the faulty BSC stating that it failed certification and should not be used until repaired.

5.2.14 Decommissioning

No biosafety cabinet should be sent to a landfill or a recycling facility as a BSC. It should be disassembled per requirements in NSF 49.

5.3 GLOVE BOXES

Glove boxes consist of a sealed chamber with glove ports and gloves for handling materials inside, a viewing window for observing, and transfer chamber or door for loading and unloading. They provide a leak-tight physical barrier between the user and the materials inside and are hence appropriate for applications that require the greatest protection against inhalation of substances used within them. Glove boxes for hazardous materials such as low level radioisotopes and carcinogens filter the chamber air prior to exhausting it through a duct system to the outside. HEPA-filtered glove boxes or Class III BSCs protect the operator and environment from hazardous airborne particulates and powders and are used to protect the user from exposure to potentially dangerous microorganisms or nanoparticles.

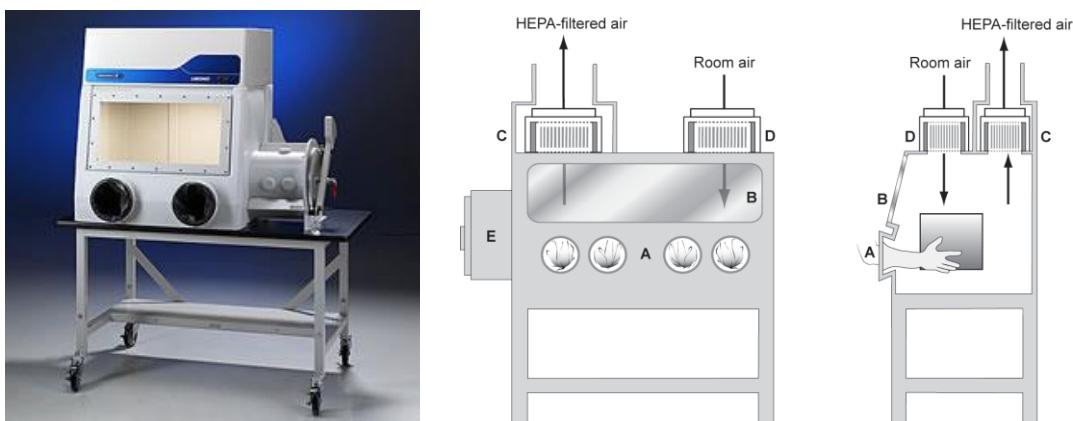


Figure 5.2 Examples of Glove Boxes

5.4 VACUUM LINE CHEMICAL TRAPS AND FILTERS

- Protect vacuum lines used to aspirate supernatants, tissue culture media, and other liquids that may contain microorganisms or hazardous particulates from contamination by the use of chemical traps and filters. These prevent contamination of the vacuum system and aerosolization of biohazardous materials or particulates into the environment.
- A chemical trap for biohazardous materials is comprised of a collection flask filled with disinfectant and overflow flask (see Figure 5.3). In addition, at BSL2 and above, an inline hydrophobic vacuum line filter meeting HEPA specifications (filtering at least 99.97% of particles greater than 0.3 μm) should be used. The use of polypropylene carboys with filling / venting closures or plastic coated heavy glass vacuum flasks are recommended to reduce the risk of breakage.

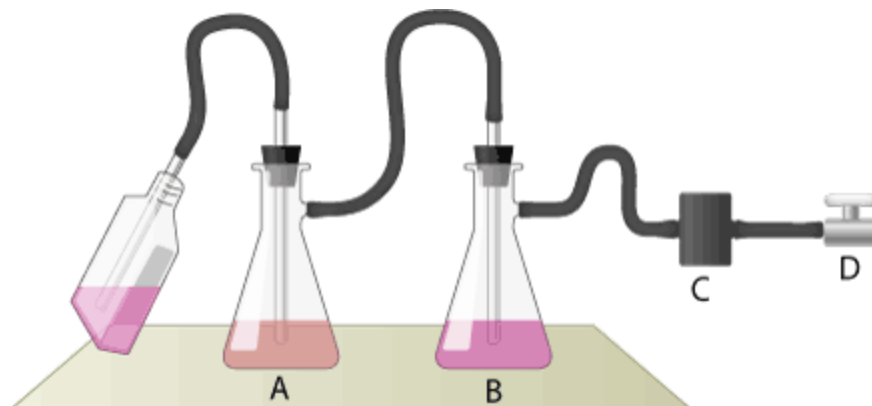


Figure 5.3 Vacuum line chemical trap for biohazardous materials. Chemical trap and filter system to protect the vacuum system during aspiration of infectious fluids. The collection flask (A) is used to collect the contaminated fluids into a suitable decontamination solution. Any overflowing liquid is collected in the connected overflow flask (B). An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.

- c. If possible locate the collection flask inside the biosafety cabinet instead of on the floor, so the liquid level can be seen easily and the flask can be emptied before it overflows. The second flask (overflow) may be located outside the cabinet, in a secondary container/tray to contain any spillage. If glass flasks are used at floor level, place them in a secondary container to prevent breakage by accidental kicking and to contain any spillage or leakage. Alternatively, both flasks can be located on the floor in a similar secondary container.
- d. Add full strength chemical disinfectant to chemical trap flasks. Allow the aspirated fluids to complete the dilution to the working concentration (e.g. start with 100 ml of a 10x strength of an appropriate disinfectant, aspirate 900 ml fluids to achieve a 10% final solution).

Replace vacuum line filters when clogged or when liquid is contacted. Used filters shall be discarded as biohazard waste.

5.5 CHEMICAL FUME HOODS

One of the most common pieces of containment equipment in the laboratory is the fume hood. The fume hood protects the worker from chemical exposure and keeps toxic / irritant vapours out of the general working area in the laboratory. The Director/HOD/PI has overall responsibility for ensuring a system is established for the safe use of the fume hood. Hence, it is the responsibility of all users to understand the correct operation and maintenance of the fume hood.

A fume hood is essentially a ventilated box with an adjustable work opening. It is designed to evenly pull in air through the front opening while minimizing turbulence. The air exits through a dedicated exhaust duct, pulling hazardous vapours, gases, etc. away from the worker.

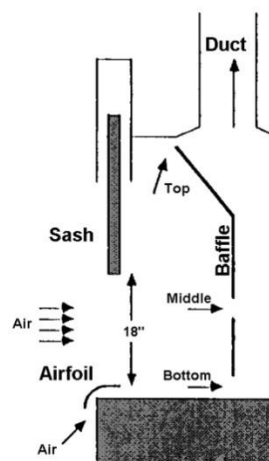


Figure 5.4 Example of chemical fume hood

5.5.1 Preparation & Material Placement

- a. Read the safety data sheet (SDS) for materials being used in a fume hood. Note and observe any precautions regarding the use of the chemical in a fume hood.
- b. Always consult the manufacturer before commissioning work involving the use of radioactive chemicals/isotopes in the fume hood.
- c. Procedures such as perchloric acid (HClO_4) digestions shall NOT be conducted in a general fume hood. Such procedures shall be carried out in a specific perchloric acid fume hood with wash down system.
- d. Do NOT use electrical-spark producing equipment in the fume hood with flammable chemicals.
- e. Check the certification label affixed on the fume hood and ensures that the fume hood has been inspected in the past 12 months by a qualified technician.
- f. Turn on the fluorescent light and hood exhaust fan 5 minutes before work begins.
- g. Confirm inward air flow by holding a tissue at the middle of the edge of the viewing panel and ensuring it is drawn in.
- h. Test the alarm if the cabinet is equipped with such device.
- i. Place equipment at least 16 cm inside the fume hood and ensure there is a 3 – 6 cm air gap around any large bulky equipment in the hood.
- j. Keep the working area of the fume hood clear of clutter and waste materials. Fume hood is *NOT* to be used as storage cabinet as it will reduce air flow and compromise fume hood extraction efficiency (see Figure 5.5).
- k. Do *NOT* store items at the back of the working area in the fume hood. This is of particular relevance where a Perspex (plexiglass) screen or lead bricks are used for radioisotope work.
- l. Do *NOT* dispose of waste chemicals via the fume hood sink as it is used mainly for rinsing and supply purposes.

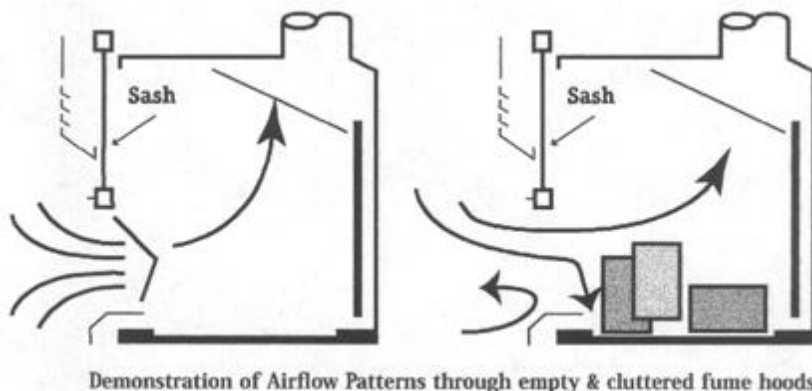


Figure 5.5 Differences of airflow patterns in empty vs. cluttered fume hood

5.5.2 Operation

- a. Ensure adequate PPE is worn (e.g. eye and face protection, laboratory apron or coat). The types of PPE required will depend on the findings obtained from the risk assessment.
- b. Fume hood users shall attend appropriate training on the safe use of the fume hood.
- c. Active work shall flow from clean to contaminated areas across the work surface.
- d. Open the sash slowly and only when required. It should be opened only as much as needed, in order to maintain effective capture of chemical fumes and to act as a safety shield to the worker.
- e. Do *NOT* put your face inside the plane of the sash when airborne contaminants are being produced.
- f. Keep traffic near the fume hood to a minimum to prevent unnecessary turbulence of the air intake.
- g. Do *NOT* leave a reaction unobserved for an extended period of time. If equipment or chemicals are to be kept in the hood longer than 8 hours, all hazardous materials must be clearly and accurately labeled. Information such as the name and contact number of the person conducting the experiment, name of the experiment, and the potential hazard of the experiment must be posted on the sash.
- h. Promptly clean up any chemical spills according to the requirements stipulated in the SDS.

5.5.3 Completion of Experiment

- a. At the end of the experiment, decontaminate the fume hood surfaces and equipment, if necessary.
- b. Return equipment and chemical bottles to their respective storage cabinets.
- c. Keep the sash fully closed when the fume hood is not in use.

5.5.4 Certification

- a. The fume hood shall be subjected to a yearly certification by a competent/qualified professional to an international standard such as "ANSI/ASHRAE 110-1995 Method of Testing Performance

of Laboratory Fume Hoods” or the “AS/NZS2243.8:2006 Safety in Laboratories - Fume Cupboards”.

- b. The PI / Laboratory Supervisor shall arrange for the yearly safety certification and keep a copy of the certification report for verification purpose.
- c. A label indicating the date of certification, the date of the next certification, to what standard test was performed, and the name of the certifier shall be affixed to the exterior of the fume hood. This label shall be provided by the certification provider of the fume hood.
- d. Re-certification is required whenever the fume hood is repaired or relocated.

5.5.5 Inspection & Maintenance

- a. The PI / Supervisor shall periodically inspect the fume hood to ensure its operational performance and observe any unsafe practices by the users.
- b. All users shall report any defects or breakdowns of the fume hood to their PI / Supervisor.
- c. Prohibit the use of faulty fume hood by proper identification like labeling the hood “out of service” or any other means.
- d. All repairs to the fume hood shall be done by a qualified technician. Any malfunction in the operation of the fume hood shall be reported and repaired before the fume hood is used again.

In general, chemical fume hoods protect the worker from chemical exposure. Air is pulled into the face of the hood and exits through a stack that exhausts to the outside of the building, moving aerosols, fumes, and vapors away from the worker. Refer to Section 7.1 of the NUS Chemical Safety Manual for more information.

5.6 CENTRIFUGES

Although it is an important tool in a research laboratory, centrifuge can also be dangerous if misused or poorly maintained. The two main hazards associated with centrifuges are from mechanical conditions and/or processing of hazardous materials.

5.6.1 Responsibilities

5.6.1.1 Principal Investigator (PI). The PI is to ensure that:

- a. Operating instructions, users’ manuals, repair and maintenance histories are available for centrifuges under his/her charge.
- b. Rotor logs are kept for high speed and ultra-centrifuges.
- c. All users are trained and competent, and training records are kept.
- d. Users observe all instructions for safe and responsible operation as detailed in this procedure.
- e. Ensure that the person who is responsible for each centrifuge carries out all the described safety & health tasks (including regular maintenance, servicing and record keeping).
- f. Appropriate warning signs are provided in rooms where potentially hazardous biological, radioactive materials, toxic or other hazardous chemicals are being centrifuged.
- g. All incidents or lapses are reported to the Faculty Safety & Health Officer.

5.6.1.2 Centrifuge operator. The operator is responsible to:

- a. Attend training identified by his/her PI.
- b. Know and follow operating instructions for rotors and centrifuges used.

- c. Safely handle, operate and clean all centrifuges and rotors during and after use.
- d. Fill out the log each time the equipment is used
- e. Report any damage to the centrifuge or rotor to the PI so that action in terms of repair or de-commissioning may be performed.
- f. Clean spills or breakage within centrifuge, noting and reporting concerns regarding possible damage, misuse, and/or failure of other users to comply with this procedure. User is recommended to follow the “best practices” on biological spill response in Section 12.3 of this manual.

5.6.1.3 Designated Person

The Head of Department shall appoint a Designated Person to take charge of the centrifuges that are part of the common facilities.

5.6.2 Classes of Centrifuges

Centrifuges are generally divided into three classes:

- a. Low Speed (up to 15,000 rpm)
- b. High Speed (15,000 rpm to 25,000 rpm)
- c. Ultracentrifuge (25,000 rpm or higher)

5.6.3 Documentation

- a. Prior to operation of any centrifuge, the user shall review the manufacturer’s manual to understand the proper operating procedures for the specific unit being operated.
- b. A copy of the manufacturer’s manual, relevant laboratory-specific SOPs or best practices should be kept in a designated location in the laboratory which is communicated to all staff members.
- c. A copy of centrifuge safety guidance should be posted next to a centrifuge for easy reference by users.

5.6.4 Safe Operation of Centrifuge

- a. The work surface under the centrifuge shall be level and firm and capable of supporting the weight of the centrifuge.
- b. Preparative centrifuge tubes made of polypropylene (sometimes polyethylene) can withstand speeds up to 20,000 rpm. These tubes should be carefully examined for stress fractures before use. A tube with a fracture may hold fluids before centrifugation, but the cracks will open under centrifugal force.
- c. Nitrocellulose tubes are inexpensive and are used for most ultracentrifugation procedures. They are intended to be used only once and then discarded. Repeated use increases the chance of tube collapse due to internal molecular stress within the tube walls. There is no way to pre-determine this, so the best practice is to always use a new tube for ultracentrifugation.
- d. Avoid overfilling tubes (or other containers used for centrifugation), as the centrifugal force generated may drive the solution up the side of the container and cause leakage.
- e. Rotors must be compatible with the centrifuge. Refer to the manufacturer’s manual.
- f. Sample loads must be balanced, swinging bucket rotors matched, and equipment must not be run with missing buckets.
- g. Ensure the spindle is clean, and that the rotor is properly seated on the drive shaft.

- h. Ensure gaskets and O-rings on the centrifuge lid, safety cups, etc. are clean, pliable and not damaged (refer to the manufacturer's manual for maintenance of these parts (i.e. appropriate cleaning solutions, application of vacuum grease, etc.). Hardened rubber gaskets with breaks in the surface will not contain potentially hazardous aerosols should a tube break during centrifugation.
- i. Lids shall be closed at all times during operation.
- j. Observe maximum speed and sample density ratings designated by the manufacturer for each rotor, and speed reductions required for running high-density solutions, plastic adapters, or stainless steel tubes.
- k. The user shall not leave the centrifuge until full operating speed is attained and the machine appears to be running safely without vibration.
- l. If vibration occurs, stop the run immediately. Wait until the rotor stops and any aerosols which may be present in the rotor or centrifuge from a broken tube (10 minutes) and then check the load balances. **Never open the lid when the rotor is moving.**
- m. In the event of a power failure, do not try to open the lid to retrieve samples for at least half an hour.
- n. Store all fixed angle vertical tube and near-vertical tube rotors upside down, with the lids or plugs removed. Swinging bucket rotors should be stored with the safety caps removed.

5.6.5 Working with Hazardous Materials

- a. Rooms where potentially hazardous biological, radioactive materials, toxic or other hazardous chemicals are centrifuged must be identified by the appropriate warning signs.
- b. When centrifuging primary human cells, human blood samples and other potentially infectious materials such as human cell lines, it shall be done at BSL2 containment level. The rotors must have aerosol, spill, or splash containment (sealed centrifuge rotors with "O-rings" or centrifuge safety cups) or be used in the biological safety cabinet. Each recipient of a human cell line from commercial sources must fully assess the potential risk of working with it in their laboratory. The risk assessment conducted must include the appropriate risk controls.
- c. Rotors shall be loaded and unloaded with the biohazardous materials in a biological safety cabinet.
- d. If centrifuging radioactive materials, keep the centrifuge behind an appropriate shield.
- e. Wait 10 minutes after the rotor comes to a complete stop before opening the lid.

5.6.6 Personal Protective Equipment (PPE)

Conduct a risk assessment and consult the best practices for spill response to determine the appropriate personal protective equipment like respirators, face shield, goggles and gloves which shall be used when handling the materials to be centrifuged, the loading and unloading of the centrifuge and spill response.

5.6.7 Emergency Procedures – Centrifuge Spill

- a. Turn off the centrifuge, notify others in laboratory, and evacuate if necessary.
- b. Post temporary a hazard warning sign.
- c. Notify PI, responsible person or departmental safety representative.
- d. Refer to the procedures for spill response in Section 12.3 of this manual and report the incident through the [NUS Accident / Incident Reporting System \(AIRS\)](#) in Section 12.4.

5.6.8 Maintenance

- a. Refer to user's manual for detailed maintenance and care of the centrifuge.
- b. Rotors and cups are to be cleaned after each use with non-corrosive (non-alkaline for anodized rotors) cleaning solutions and stored inverted when required by the manufacturer. Mild detergents are recommended to prevent damage to the rotors.
- c. After proper clean-up, rinse the rotor with de-ionized or distilled water.
- d. Wash rotor and cups or cavities with warm water and mild detergent after each use. Do not use metal test tube brushes for cleaning cups or cavities. All traces of detergent should be removed prior to air-drying.
- e. Keep rotors clean and dry. If spills occur, make sure the rotor has been cleaned and decontaminated. To prevent corrosion, do not expose aluminum rotor components to strong acids or bases, alkaline lab detergents, or salts (chlorides) or heavy metals (e.g. cesium, lead, silver or mercury).
- f. Avoid mechanical scratches. The smallest, scarcely visible scratch allows etching to enlarge the fracture, which is subject to enormous rupturing forces at high "g" – a vicious cycle leading to rotor explosion.
- g. Rotors shall be retired after the manufacturers' recommended revolutions or years of service, whichever comes first, except where an annual stress test (magnaflux or other professionally recognized analysis) proves an absence of structural flaws.
- h. If a centrifuge is experiencing problems or maintenance is required, the unit must be immediately taken out of service, disconnected from the power source and clearly marked DO NOT USE until serviced. This notice will include the name of the person, the date, the reason and the signature of the PI or the Responsible Person.

5.6.9 Records

Rotor Log is to be kept for one year. Some newer equipment may have data-logging capability. Consult the manufacturer's instructions for specific recordkeeping requirements.

5.7 OTHER SAFETY EQUIPMENT USED IN A BIOMEDICAL LABORATORY

- 5.7.1 Sharps containers or devices with engineered sharps injury prevention features** are engineering controls used for safe handling of sharps for prevention of percutaneous injuries. See Section 7.5 of this manual for best practices when handling sharps.
- 5.7.2 Pipettes and pipetting aids** are used for transferring and measuring fluids that may contain hazardous agents (i.e. chemical, toxic, corrosive, radioactive or biohazardous agents). These were developed and have become the standard for such manipulations after laboratory acquired infections resulted from oral aspiration of infectious materials, mouth transfer from a contaminated finger or inhalation of aerosols. See Section 7.14 of this manual for more details.

CHAPTER 6 ADMINISTRATIVE CONTROLS

Administrative controls are important in laboratory safety because they stipulate behaviours and actions that apply to and protect all members of the laboratory staff. They are also an important means of evaluating compliance with regulatory requirements.

Administrative controls include:

- placement of proper signs and labels in and about the laboratory
- medical surveillance programmes
- training
- laboratory inspections
- development of standard operating procedures
- establishment of sound safety attitudes.

6.1 SIGNS AND LABELS

A biohazard label is required for all areas or equipment which contain biological agents or toxins.

6.1.1 Door Signs

- a. Each laboratory and animal facility must have a sign at the entrance of the room that provides safety information to visitors and service personnel.
- b. The door signs should be affixed with the door sign displaying all the appropriate hazard signs that are present in the laboratory, which can be generated using the [Standard Lab Sign Posting Generator](#) software in OSHE's website.
- c. The door sign shall be printed in colour on a standard A4 sized paper. The sign must be clear and can be seen clearly from a distance of 5 meters away.
- d. The sign has to be posted at least 1.5 meters from the floor either on the door to the lab or at the side wall next to the door provided that the sign is not more than 500 mm from the door. If the lab has more than one door, all doors must also be sign posted.
- e. The sign must be enclosed in a transparent plastic folder or laminated. The sign must be firmly affixed on the door through suitable means.
- f. Entrance to laboratories that handle Risk Group 2 agents, human blood or other potentially infectious materials must be posted with a BSL2 biohazard sign that contains the universal biohazard symbol, the legend "Biohazard" and the term "Biosafety Level 2".



Figure 6.1 Biohazard sign with Biosafety Level 2 designation

6.1.2 Internal Lab signs

- a. Room signs must contain information on all laboratory hazards in use within the laboratory (carcinogens, acutely toxic agents, biological agents and toxins, radioactive materials, etc.), specific personal protective equipment (PPE) needed in the lab, as well as the name and phone numbers of the principal investigator or other responsible person(s).
- b. Biological hazard warning labels must be used to identify infectious waste containers, containers for storage of infectious materials as well as refrigerators, incubators and/or freezers where biological agents and toxins are stored. Large pieces of equipment for handling such materials (e.g. centrifuges, biological safety cabinets, etc.) must be similarly labeled.
- c. Certain areas and pieces of equipment within a laboratory may also require signs. Refrigerators, freezers, cabinets and other storage facilities require the biohazard symbol whenever used to store infectious agents of Risk Group 2 or higher or human blood or blood products, unfixed tissues, cell or organ cultures, body fluids or excreta.
- d. Any hazard sign posting or PPE sign posting must be placed at the entrance of the area where hazards have been identified.
- e. The sign posting must be placed in a prominent position at height not less than 1.5 meter above the floor. The sign should be of good construction and firmly affixed to the intended location. It should also be clearly identified by laboratory users.
- f. The sign posting must be reviewed at least every year or when the scope of work changes within the laboratory that necessitates a new risk assessment exercise be done or reviewed
- g. First time laboratory users or visitors must be briefed on the meaning of the signs in the laboratory.

6.2 MEDICAL SURVEILLANCE

The NUS Occupational Health Programme aims to protect and promote the health and well-being of NUS staff and students at work. Medical surveillance programs are provided for personnel who are at risk of occupational exposure to materials of animal or human origin and infectious disease agents.

- Staff and students will be in contact with human blood, tissues, etc. must undergo hepatitis B screening and vaccination.
- Staff and students conducting animal research must undergo tetanus vaccination.
- Staff and students conducting research on materials containing Risk Group 2 and above agents must undergo periodic medical examinations based on the risk assessment of the research protocol. Specific serological tests and immunizations may be required.
- Staff or students conducting deliberate research in BSL2 or 3 or ABSL2 or 3 laboratories or working within such facilities, should undergo periodic medical examinations to assess fitness to work with infectious disease agents or animals.

Application forms and more details on the [Occupational Health Programme](#) are available at OSHE website.

6.3 TRAINING

Good microbiological and laboratory practices are essential for a safe work environment. The purpose of training is to provide the understanding, technical knowledge, and tools to the laboratory personnel to improve his or her daily laboratory safety practices.

Under the University's [Structured Safety Training System](#) (SSTS), it is mandatory for all PIs and staff working in laboratories to undergo safety trainings based on their job scope and work hazards. Depending on the structure and content of the safety trainings, they will be available through e-learning in IVLE or will be provided by instructors in training rooms. The trainings will be competency based and will be managed by OSHE through IVLE and also with support from Office of Human Resources (OHR). Refresher training course has to be taken every two years.

All laboratory personnel who are dealing with biological hazards are required to attend the Biological Safety course. OSHE also provides courses for the chemical and radiological safety programmes. For work with vertebrate animals, all personnel are required to undergo the "Responsible Care and Use of Laboratory Animals (RCULA)" Course and/or the "Responsible Care and Use of Fish (RCUF)" Course.

At the minimum, all personnel working with biological materials should be trained in the following areas prior to the start of their experiments:

- Knowledge of the NUS Laboratory Biorisk Management Manual
- Experimental procedures to be used
- Decontamination and spill cleanup procedures
- Safe handling methods for any infectious agent and/or recombinant DNA (rDNA) they might be handling
- Proper methods for transporting biological agents and toxins

In addition, they should receive adequate laboratory specific training from the Principal Investigator on:

- Good laboratory and animal practices as applicable
- Site specific information on risks, hazards and procedures
- Laboratory or environment specific BSL2 or 3 procedures, as applicable

The PI is responsible for ensuring that laboratory personnel in his/her laboratory receive proper training in the biological agents and toxins and controls specific to his or her laboratory and the safe conduct of the experimental procedures to be used.

6.4 INSPECTIONS

PIs should develop a system for internal audits to ensure that they have an appropriate safety management system in place for a safe laboratory working environment.

PIs may refer to the following checklists:

- [Internal Audit Checklist](#)
- OSHE's generic checklist, [NUS Self-Help Safety + Health Starters' Checklist - Part I](#)
- Checklist based on WHO [Laboratory Biosafety Manual, 3rd Edition](#) (see PART VIII. Safety checklist)

Inspections and audits are also carried out periodically at the level of the Faculty Safety Officer, Departmental Safety Committee, Faculty Safety Committee or OSHE.

6.5 STANDARD OPERATING PROCEDURES (SOPS)

Standard Operating Procedures (SOPs) are developed to establish a consistent, repeatable method for performing common, repetitive tasks. Such tasks have been performed many times and most of the common errors and unsafe practices have been discovered and corrected. The set approach and methods presented in the SOP ultimately enhance both the efficiency and the safety of the procedure.

SOPs should be kept updated. Laboratory personnel should be alert for ways to improve the SOPs in use and to ensure that demonstrated improvements are incorporated into the documents.

CHAPTER 7 GOOD MICROBIOLOGICAL TECHNIQUES & RECOMMENDED WORK PRACTICES

Human error, poor laboratory techniques and misuse of equipment cause the majority of laboratory injuries and work-related infections. Training, experience, knowledge of the agent and procedural hazards, good habits, caution, attentiveness, and concern for the health of co-workers are prerequisites for laboratory personnel in order to reduce the inherent risks of working with hazardous agents. This chapter provides a compendium of methods, recommended work practices and technical procedures that are designed to avoid or minimize the most commonly reported hazards.

7.1 PRUDENT PRACTICES AND GOOD TECHNIQUE

- a. Human factors and attitudes are important elements for considerations of biosafety in the laboratory. Factors compromising safety include:
 - The lack of accident perception
 - Inflexibility of work habits, that tend to preclude preventative action when an accident situation is recognized
 - Working too rapidly
 - Intentional violations of regulations
 - Performance of routine procedures such as diluting and plating cultures is the most frequent task being performed at the time of laboratory accidents.
 - Working when one is very tired
 - Working at a disorganized and crowded laboratory bench
- b. Each lab person working with biological agents and toxins must be aware of the importance of the proper attitude in preventing accidents in the laboratory.
- c. Prudent practices and good technique are of primary importance in laboratory safety. Both are based on sound technical knowledge, experience, common sense and an attitude of courtesy and consideration for others.
- d. At a minimum, the Seven Basic Rules of Biosafety should be the basis of any personal laboratory work ethic:
 - **Do not mouth pipette.**
 - Manipulate infectious fluids carefully to avoid spills and aerosol production.
 - Use needles, syringes and other "sharps" carefully to avoid self-inoculation; and dispose of sharps in puncture-resistant and leak-proof containers. Do not recap syringes. Where possible, replace the use of sharps with other alternatives to avoid potential needlestick injuries.
 - Use personal protective equipment such as laboratory coats, gloves and eye protection.
 - Wash hands following all laboratory activities after the removal of gloves, and immediately after contact with infectious materials.
 - Decontaminate work surfaces before and after use and immediately after spills.
 - Do not eat, drink, store food, apply cosmetics or smoke in the laboratory.

7.2 HOUSEKEEPING AND PERSONAL HYGIENE

Well-defined housekeeping procedures and schedules are essential in reducing the risks associated with working with pathogenic agents and contribute to safe research programme.

Injuries and exposures are more likely to occur in poorly maintained, disorderly work areas than in neat, well-kept spaces. For those with the luxury of unshared work space, personal safety is greatly enhanced by keeping that space neat, clean and orderly. More often than not, work space is shared with others and good personal housekeeping in the laboratory becomes a cardinal rule. Leaving behind a mess after work exposes others to risks of which they may have little or no knowledge. In shared spaces, consideration for others and cleaning up after oneself is essential for maintaining a safe working environment.

7.2.1 Objectives of Housekeeping

- a. To provide an orderly work area conducive to the accomplishment of the research programme.
- b. To get rid of physical clutter that could interfere with the activities of laboratory personnel at a critical moment in a potentially hazardous procedure.
- c. To provide a work area that is free of injury arising from physical hazards or background contamination.
- d. To prevent the accumulation of materials from current and past experiments that constitutes a hazard to laboratory personnel.
- e. To ensure that locations of various hazards will be known.
- f. To prevent the creation of aerosols of hazardous materials
- g. To prevent the accumulation of organic debris that may:
 - harbour microorganisms that are potentially threats
 - enhance the survival of microorganisms inadvertently released in experimental procedures.
 - retard penetration of decontaminants
 - be transferable from one area to another on clothing and shoes
 - with sufficient buildup, become a biorisk as a consequence of secondary aerosolization by personnel and air movement
 - cause allergenic sensitization of personnel (e.g. to animal dander)

7.2.2 Housekeeping Procedure

- a. Housekeeping tasks should be carried out by lab personnel on an individual basis for their immediate work areas and on a cooperative basis for areas of common usage.
- b. Housekeeping chores, both individual and cooperative, should be performed on a periodic basis. Routine housekeeping will provide a work area free of significant sources of background contamination.
- c. Areas for which housekeeping should be carried out include but are not limited to:
 - ♦ Corridors ♦ Lab Entrances and Exits ♦ Aisles ♦ Work Benches ♦ Floors ♦ Lab Equipment Clean-up ♦ Biological Safety Cabinets ♦ Lab Reagents Storage Areas ♦ Refrigerators ♦ Cold Rooms ♦ Deep Freezers ♦ Incubators ♦ Waste Storage Areas ♦ Cryogenic Tanks ♦ Work Surfaces ♦ Lab SOPs
- d. Housekeeping tasks should be assigned to personnel who are knowledgeable of the laboratory environment.

- e. Keeping a schedule of housekeeping tasks helps to ensure that work in the laboratory will not be interrupted.
- f. PIs should carry out periodic inspections of the lab to assure compliance.

7.2.3 Good Housekeeping Practices

- a. All areas of the laboratory must be kept clean and orderly. Keep the area as clean as the work allows throughout the day and all working surfaces should be decontaminated and cleaned at the end of each work day.
- b. Stock solutions of disinfectants e.g. 70% ethanol, 10% bleach should be maintained at each bench top and biological safety cabinet work area. Storage of stock or working solutions of bleach in open containers, particularly at high temperatures, releases chlorine gas thus weakening their germicidal potential. As a general guide, solutions receiving materials with high levels of organic matter several times a day should be changed at least daily, while those with less frequent use may last for as long as a week.
- c. Shared workbenches or lab space should be cleaned prior to leaving it for the next user as a common courtesy.
- d. Keep floors clean and free of tripping hazards or clutter.
- e. Keep stairways, hallways, passageways / aisles and access to emergency exits dry and free of obstructions.
- f. Store items so they do not block access to the fire extinguisher(s), safety equipment, electric panel boxes, or other emergency items such as an eyewash or safety shower.
- g. Do not allow combustible material such as paper, cardboard boxes, or pallets to accumulate. Do not place these materials in hallways.
- h. Minimize extraneous supplies and equipment. To the extent possible, restrict all work areas to only those items needed for the immediate experimental procedure.
- i. Do not clutter fume hoods or biosafety cabinets with unnecessary items. The safety of these workspaces and the ventilation provided is compromised when excessive items and equipment are kept in this space.
- j. Label all personal materials clearly for ease of identification.
- k. Do not let materials accumulate. Dispose of materials, chemicals, and equipment that are no longer needed.
- l. Store chemicals in designated locations - store flammable liquids in a flammable liquids cabinet. Do not store acids above shoulder height or in unprotected metal cabinets. Store water reactive materials away from water sources, such as sprinkler systems and sinks. Chemical products should be returned to their proper place after use (see Section 7.5 of the NUS Laboratory Chemical Safety Manual).
- m. Maintain an inventory of hazardous (biologicals, chemicals, etc.) agents. Store only the amount of material reasonably needed. Do not over-purchase.
- n. Do not store frequently used or heavy items on top shelves. Locate supplies used daily close to the work area and place items used periodically in nearby storage areas.
- o. Shelves should be equipped with doors or lips to prevent items from falling.
- p. Keep an adequately stocked spill kit in the work area. Clean up all small spills immediately. Know what to do in the event of a hazardous material spill and take appropriate action immediately.
- q. Always restrain compressed gas cylinders with chains or ropes.
- r. Dispose of all laboratory wastes (e.g. radioactive, chemical, biological and sharps wastes) properly. Ensure waste containers are placed near the point of use and are of adequate size. Do not over fill the collection containers.

- s. Decontaminate all contaminated plasticware / glassware prior to washing or disposal (see Chapter 8 of this manual for details).
- t. Periodic inspections should be carried out by the PI.

7.2.4 Personal hygiene

- a. Personal hygiene is an important means to enhance personal protection in the laboratory.
- b. Appropriate personal protective equipment such as lab coats and gloves must be worn in the laboratory work areas and removed prior to leaving the laboratory after lab activities. Do not wear contaminated or potentially contaminated laboratory coats outside the laboratory.
- c. All laboratory coats should be laundered regularly by sending to a laboratory coat laundering service. Do not bring laboratory coats home to wash!
- d. Wash hands with soap and water immediately after removing gloves or after contact with biohazardous / infectious agents. This ensures that any contamination of the hand through breaches in the integrity of the glove or during the process of glove removal is washed away before the contamination can be spread to environmental surfaces.
- e. Do not eat, drink or smoke in the laboratory. Do not store food or drinks in laboratory areas such as cold rooms or laboratory refrigerators.
- f. Do not perform personal cosmetic tasks such as applying makeup, manipulating contact lenses, trimming fingernails, or combing hair. These activities provide opportunities for exposure to infectious agents.

RECOMMENDED WORK PRACTICES

Recommended work practices detailed in the following sections are aimed at providing guidance for the use and manipulation of biological agents and toxins commonly found in laboratories where biological work is performed.

7.3 SAFE HANDLING OF BIOLOGICAL MATERIALS

Major biohazards associated with laboratory work are infections with pathogenic micro-organisms such as viruses, parasites or bacteria that are either being studied in the laboratory, or present as contaminants in blood, serum, body fluids, cell lines and tissue samples under analysis.

Improper storage, handling or transport of biological materials in the laboratory poses a risk of infection to the personnel involved and others who may come in contact with the personnel or a contaminated environment.

7.3.1 Definitions

Biological materials – any one type or combination of live, frozen or lyophilized material that includes the following:

- Cell lines derived from human, non-human primates, mammalian sources
- Tissues, fluids, etc. from human, non-human primates, mammalian
- Recombinant DNA from animal or plant sources

- Bacteria
- Virus
- Fungi, parasites, insects, or other living material
- Prions

Biohazardous - possessing the potential to produce an infection or genetic alteration in humans, animals or plants.

Biohazardous materials - may include, but are not limited to, bacteria, fungi, viruses, parasites, recombinant products, allergens, cultured human or animal cells and the potentially infectious agents these cells may contain, such as viruses, prions and other infectious agents.

Biohazardous materials do not include live animals that are healthy and presumed to be non-infectious, but do include unhealthy animals that are known or suspected to be infectious.

Biological materials are not considered to be potentially hazardous when they have been chemically, thermally, or otherwise treated to render them permanently non-viable / permanently inactivated / permanently incapable of insertion or infection into living cells.

7.3.2 Risk Assessments

Evaluate the risks associated with **all** materials to be handled. Consider:

- the species of the source cells, the tissue or cell type origin (i.e. all established or permanent cultures of human lymphocytes should be handled on the assumption that they harbour the Epstein-Barr virus)
- the culture type
- the intrinsic properties of the cell culture
- subsequent properties acquired as a result of any genetic modification
- the possibility that the cell culture may inadvertently or deliberately become contaminated
- appropriate use, storage, decontamination, disposal, and emergency response procedures

7.3.3 Containment Level

No cell line or biological material is guaranteed to be non-hazardous. As a rule, for cell cultures or biological materials known to harbor an infectious etiologic agent, manipulate the cultures in compliance with containment measures recommended for the etiologic agent. Appropriate biosafety level (refer to Section 4.4 & 4.6) and use of BSC (refer to Section 5.2) should be employed whenever applicable. Please refer to **Appendix A.1** for guidance on assignment of containment level.

If your lab involves research using human blood, human cell lines (even commercial, established human cell lines), or human tissue cultures you must employ at least **Biosafety Level 2 (BSL2)** practices and procedures.

Handle all human samples such as blood or tissues as if they are infectious, even if they are considered non-infectious. Human samples include: human blood, blood products, certain body fluids (semen, vaginal, cerebrospinal, synovial, pleural, peritoneal, pericardial, and amniotic), and any body fluids in which visible blood is present, and any unfixed human tissue or organ.

This is consistent with the recommended practices in CDC / NIH publication, [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)](#), 5th edition and the World Health Organization publication, [Laboratory Biosafety Manual](#), 3rd Edition.

7.3.4 Occupational Health Requirements

Under the NUS Occupational Health Programme, laboratory personnel working with materials of human origin are required to have immunization against Hepatitis B, whereas personnel working with animals are required to be immunized against tetanus (see Section 6.2, Medical Surveillance).

7.3.5 Receipt

- a. Personnel who receive and unpack biological materials should be aware of the potential health hazards involved and the appropriate safety precautions to be taken, particularly when handling materials derived from human or primates.
- b. Before opening any package, read the safety information that usually accompanies the commercially available products e.g. Safety Data Sheets / product information sheets for cell lines and microbial cultures. If in doubt, only open the package in a biological safety cabinet.
- c. Wear disposable gloves and a lab coat/gown during receipt of biological materials.
- d. Keep supplies of an appropriate disinfectant available on hand.
- e. Check for any leakage, cracks or breaks in the containers.
- f. Wash your hands thoroughly with soap after handling.

7.3.6 Storage

All biological materials to be stored must be clearly labeled with the scientific name, and/or descriptions, date of storage and name of person who stored them to facilitate identification. Autoclave and discard all unlabeled and obsolete items (this is especially important when personnel leave a research group).

Expired and other unwanted material must be decontaminated properly.

Hazard warning signs (such as shown below), indicating the biosafety level of the biological material being used, must be posted on laboratory doors, cold rooms, refrigerator / freezer doors and cryogenic tanks.



Figure 7.1 Example of biohazard sign with Biosafety Level 2 designation

Keep and maintain an inventory of biological materials stored in refrigerators, freezers and cryogenic tanks.

Regularly clean and disinfect refrigerators etc. in which biological cultures are stored.

7.3.6.1.1 Storage Containers

Storage containers must be robust and leak-proof. Visually inspect to ensure that no material remains on the outside of the container.

Product specification forms that accompany the biological materials should not be wrapped around the containers but removed and filed appropriately. If they are to accompany the biological material for example, during transport, place them in separate, preferably waterproof bags / envelopes.

If a biohazardous material is stored in a refrigerator or cold room, place it in a secondary container large enough to contain the contents should the container break.

All secondary containers (plastic bags, bottles, boxes, bins, etc.) used to store biological materials must also be labeled clearly with the universal biohazard symbol as shown below. Biohazard labels for secondary containers can be obtained from OSHE.



Figure 7.2 Example of a generic biohazard sign

Decontaminate all containers used for storage before reuse or dispose of them properly as potentially biohazardous.

7.3.6.1.2 Storage of Ampoules Containing Infectious Materials

Storage of ampoules / cryovials in liquid phase nitrogen allows the lowest possible storage temperature to be maintained with absolute consistency but creates potential hazards. If they are cracked or imperfectly sealed, contamination may occur via the liquid nitrogen. They may also break or explode on removal due to a build-up of excessive pressure during thawing. For these reasons, ampoules containing highly infectious materials should never be immersed in liquid nitrogen and preferably should be non-glass materials.

If very low temperatures are required, store the ampoules in the vapour phase of liquid nitrogen or deep freezers.

Wear full personal protective equipment (laboratory coat, fully covered shoes, insulated gloves and full-face shield) when removing ampoules from cold storage. Disinfect the outer surfaces of ampoules when they are removed from storage.

7.3.7 Transport of Biological Materials

This section is covered in detail in Chapter 9 on “Transport of Biological Materials”. In summary:

- a. **For transport within the University:** Spills or leakage should be prevented via the use of appropriate sealed and leak-proof primary, secondary and tertiary containers and items to be transported are to be labeled with essential information.
- b. **For import or transport within Singapore:** procedures are governed by the Biological Agents & Toxins Act (BATA) under the Ministry of Health for human pathogens; or the Animal and Birds Act under the Agri-Food and Veterinary Authority for zoonotic pathogens.
- c. **For export:** Export of high-risk biological agents and toxins is controlled by the Strategic Goods Control Act under the Singapore Customs.
- d. **For package and labeling:** Biological agents to be exported must be packaged and labeled according to International Air Transport Association (IATA) standards.

7.3.8 General Safety Procedures for Handling of Infectious Materials

Whenever work with infectious agents is performed, all appropriate measures must be taken to protect laboratory personnel and the environment. Safety measures to take include the use of engineering and administrative controls, and personal protective equipment. But the most important element of containment is prudent laboratory practices, and standard microbiological techniques as detailed below.

7.3.8.1 Avoiding Dispersal of Infectious Materials by Aerosol Production

Aerosols are dispersions of airborne liquid or solid particles created by most laboratory manipulations. Exposures to microbial agents through inhalation or contamination of environmental surfaces are major routes of infection in laboratory acquired infections.

Examples of aerosol-producing activities in the laboratory:

- shaking or vortexing tubes, stirring
- sonicating, homogenizing, blending, grinding, cell disruption with French press
- opening lyophilized cultures, opening snap top tubes, opening ampoules
- breakage of culture containers
- flaming loops or slides
- pouring liquids

- centrifugation steps e.g. Opening/ filling centrifuge tubes, removing supernatant, resuspending pellets, centrifugation itself and breakage of tubes during centrifugation
- blowing out pipettes
- pulling needles out of septums, filling a syringe
- intranasal inoculation of animals
- cage cleaning, changing animal bedding
- harvesting infected material from animals, eggs, and other virology procedures

Use measures that avoid the creation of an aerosol, reduce the extent of aerosol formation, or contain the aerosol away from personnel and general air circulation in the laboratory. Such measures would include conducting procedures that may produce infectious aerosols (e.g. agitating, blending, grinding) in a **biological safety cabinet** or similar containment device.

Vortexing / Shaking / Stirring - Keep containers properly sealed. Vigorous shaking will create a heavy aerosol. To resuspend liquids e.g. cultures, use a swirling action to create a homogeneous suspension with a minimum of aerosolization. Wait a few minutes after the procedures before opening the container.

Blending - Use safety blenders, although expensive, are designed to prevent leakage from the blender jar. Household blenders can leak and therefore do not prevent the spread of aerosols. Operate blender and similar equipment in the biological safety cabinet whenever possible. See also Section 7.7 “Blending, Mixing, Sonicating, Cell Disruption Equipment”.

Opening culture tubes, plates, bottles and flasks - Immediately following shaking or centrifugation, allow aerosols to settle for 5 to 10 minutes before opening the containers. Manipulate slowly as films of liquid or dried infectious material may collect at or near the rim / neck of containers and may be dispersed into the air when disturbed. Particular care is required when opening plates, tubes, or bottles containing fungi, as the operation may release a large number of spores. Such cultures should be manipulated in a biological safety cabinet.

Opening ampoules – Always open ampoules in a biological safety cabinet to control any aerosol produced. To open a sealed-glass ampoule, nick the neck of the ampoule with a file, hold the ampoule upright in a disinfectant-soaked paper towel to protect hands, and snap it open at the nick. Reconstitute the contents of the ampoule by adding liquid slowly to avoid aerosolization of the dried material. Exercise caution as sealed glass ampoules stored in liquid nitrogen can explode on removal dispersing materials into the atmosphere. Use alternatives e.g. polypropylene tubes / cryovials if possible to eliminate this hazard.

Pouring of liquids - Never pour or decant liquids containing infectious agents e.g. bacterial cultures, virus suspensions. Aspirate off supernatants or use a mechanical pipette.

Pipetting - Pipettes are used for volumetric measurements and transfer of fluids that may contain infectious, toxic, corrosive or radioactive agents. Laboratory-associated infections have occurred from oral aspiration of infectious materials, mouth transfer via a contaminated finger and inhalation of aerosols. Exposure to aerosols may occur when liquid from a pipette is dropped onto the work surface, when cultures are mixed by pipetting, or when the last drop of liquid is blown out. A pipette may produce and amplify hazards if improperly used. The following safe pipetting techniques are required to minimize the potential for exposure to biologically hazardous materials:

- Never mouth pipette.** Always use a pipetting aid.

- b. If working with biohazardous or toxic fluid, confine pipetting operations to a biological safety cabinet.
- c. Always use cotton-plugged pipettes when pipetting biohazardous or toxic materials, even when safety pipetting aids are used.
- d. Do not prepare biohazardous materials by bubbling expiratory air through a liquid with a pipette.
- e. Do not forcibly expel biohazardous material out of a pipette.
- f. Never mix biohazardous or toxic material by suction and expulsion through a pipette.
- g. When pipetting, avoid accidental release of infectious droplets. Place a disinfectant soaked towel on the work surface and autoclave the towel after use.
- h. Use "to deliver" pipettes rather than those requiring "blowout".
- i. Do not discharge material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.
- j. Place contaminated, reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them. Do not place pipettes vertically into a cylinder. Autoclave the pan and pipettes as a unit before processing them as dirty glassware for reuse (see Chapter 8, Decontamination and Disposal).
- k. Discard contaminated disposable pipettes in an appropriate sharps container. Dispose of the sharps container as infectious waste according to the Departmental procedures for disposal of biohazardous waste.
- l. Place pans, or sharps containers for used, contaminated pipettes inside the biological safety cabinet to minimize movement in and out of the BSC. Close the container, disinfect the outside surface, and remove from the BSC.

Centrifugation - Use containment devices (e.g. BSCs, sealed canisters, safety cups or buckets with covers, sealed tubes or sealed rotors, etc.). Allow aerosols to settle for 10 minutes before opening a centrifuge (see Section 5.6 for more details on centrifuge safety).

Lyophilizing - If possible, load samples in a biological safety cabinet. Use a vacuum line trap or HEPA filter to protect the lyophilizer vacuum pump exhaust. Disinfect all surfaces of the unit that have been exposed to the agent after lyophilization (see Section 7.11 for more details.)

Sterilizing inoculating loops/needles - Use an electric a hooded Bunsen burner, shielded micro-incinerator or glass bead sterilizer. If flaming the loop, avoid splattering by gradually introducing the inoculating loop into the Bunsen burner; and allowing inoculating loops or needles to cool before touching biological specimens. Alternatively, use disposable sterile plastic loops and needles (see Section 7.8 for more details).

Disinfecting spills - If a spill occurs that may generate aerosols, leave the area, close the door, and wait 30-60 minutes to allow dissipation of aerosols. Do not spray disinfectant onto liquid spills, as this will create aerosols. Instead, cover the spill with paper towels and gently pour liquid disinfectants to the affected area, proceeding from the outer edge of the spill to its center and. Let it soak for 30 minutes (refer to "Biological Spill Response" in Section 12.3 for detailed procedures).

7.3.8.2 Avoiding Contact of Infectious Materials with Skin and Eyes

Infectious agents can be introduced to the mucous membranes of eyes via splashes, splatters or contact with contaminated fingers or other objects. These agents include viruses and bacteria that can cause conjunctivitis (e.g. adenovirus, *Herpes simplex*, *Staphylococcus aureus*, etc.) and viruses that

can cause systemic infections, including bloodborne viruses (e.g. Hepatitis B and C viruses, human immunodeficiency virus, etc.), herpes viruses, and rhinoviruses. Infectious agents can also be introduced via non-intact skin (e.g. small cuts, open wounds, etc.) from hands that have been in contact with a contaminated surface (i.e. benches, phones, computers, equipment handles, etc.) or by failure to wash hands after handling such agents.

Wear appropriate personal protective equipment (laboratory coats, gloves, eye protection, etc.) at all times when handling infectious materials (refer to guidelines on selecting appropriate PPE in Chapter 11 for more details).

Avoid touching any part of your body (skin, face, eyes, mouth, etc.) with your gloved hands as infectious materials deposited on your hands can come into contact with the area as a result.

Always remove personal protective equipment and wash your hands with soap and water before leaving the laboratory work area and other restricted areas. Do not wear your laboratory coat or gloves outside of the laboratory into public areas.

Wear proper covered footwear in the laboratory. Do not wear open-toed or cloth shoes or sandals in the laboratory.

Cover up any open wounds or cuts with a plaster before starting work in the laboratory.

Do not use gloved hands to open doors or handle shared items (e.g. phones, computers, etc.) that would be touched by someone not wearing gloves.

Replace disposable gloves as soon as possible if contaminated, torn, punctured or damaged in any way.

Hands should be washed immediately with soap and water, after removing gloves, upon completion of any procedure in which infectious material is used.

Do not handle contact lenses or apply cosmetics in the laboratory.

Wear suitable eye protection when performing procedures which may result in the splashing of potentially infectious materials. Consider wearing a respirator when performing aerosol-generating procedures, especially materials known to be transmitted through inhalation.

7.3.8.3 Avoiding Injection of Infectious Materials

Infections can occur through intact skin via percutaneous / sharps injuries causing accidental inoculation of infectious materials (see “Safe handling of sharps” in Section 7.5 for safety precautions).

7.3.8.4 Avoiding Ingestion of Infectious Materials

Do not eat, drink or smoke in the laboratory or place any articles e.g. pen or pencil in your mouth.

Do not store food or drinks in the laboratory premises, cold rooms, laboratory refrigerators, or equipment. Similarly, do not bring biological materials into any premises where food and drinks are consumed or stored.

Do not apply cosmetics in the laboratory.

Wear a face shield or surgical mask when performing procedures which may result in the splashing of potentially infectious materials.

NEVER pipette any materials by mouth. Use a mechanical pipetting device (see Section 7.14).

7.3.9 Recommended Work Practices for Cell Cultures

7.3.9.1 Good Microbiological Practices

- a. Be trained in aseptic techniques. Contamination of cell cultures with pathogenic micro-organisms or cross-contamination with other cells can be significantly reduced or even prevented by adhering to safe, sterile working practices.
- b. Avoid the use of sharps whenever possible.
- c. Sterile handling should be carried out in a BSC (refer to Section 5.2).
- d. Use of proper PPE.

7.3.9.2 Cell Lines

- a. Use well characterized cell lines where possible. Take into consideration the trustworthiness of the source.
- b. Read all information such as Safety Data Sheets provided with the cells and ensure that you are aware of the hazards and risk controls for handling.
- c. NEVER handle or manipulate cells derived from yourself. Autologous cells, if accidentally reintroduced, will express the tissue type of the operator and hence could evade normal immune responses that recognize and destroy foreign cells. The closer the cells are phylogenetically related to humans, the greater the potential risk of contamination (highest to lowest risk: human autologous > human heterologous > non-human primates > other mammalian sources > avian > invertebrates).
- d. The tissue from which the cells originate affects the sensitivity to possible infection (highest to lowest risk: hematogenous (blood & lymphoid tissue) > neural tissue > endothelium > gut epithelium > epithelium > fibroblasts).
- e. The culture types, in order of decreasing risk, are primary cell cultures, continuous cell lines (immortalized cells), intensively characterized cells, including human diploid fibroblasts (e.g. WI-38, MRC-5, and IM90).
- f. When using viral sequences in recombinant cell lines, the transfer of pathogenic functions or virulent factors and the activation of endogenous viruses should be considered. In some cases, although the viral expression systems (e.g. adenoviral and retroviral) are disabled by replication deficiency, replication-competence virus may still arise in cultures and therefore must be monitored.
- g. Treat cell cultures from undefined / uncontrolled sources as Risk Group 2 agents and handle them as if they are infectious. If there is a reasonable likelihood of adventitious agents of higher risk class, e.g. cell lines from patients at risk of HIV, the cell line should be handled under appropriate containment level until tests have proven safety.
- h. It is advisable to quarantine new cell cultures brought to the laboratory until the culture does not show growth of contaminating bacteria, fungi, or mycoplasmas. It is noteworthy that these organisms not only survive in the tissue culture environment but also on work surfaces to some extent.
- i. Work with one cell line at a time and disinfect the work surfaces between two handlings involving different cell lines.

- j. Routinely carry out quality control checks of cells (e.g. checks for mycoplasma contamination) to ensure the absence of likely contaminating pathogens.

7.3.9.3 Culture Media

- a. When preparing media to be used with more than one cell line, aliquot the media into separate containers for each cell line. This will reduce the chances of contamination.
- b. Restrict use of antibiotics in growth media to reduce the chance of a resistant agent from growing. Rely on good aseptic technique rather than on antibiotics to keep cell lines free of contamination.
- c. Obtain animal sera from a reputable source as serum can be contaminated with adventitious bovine viruses or prions e.g. the agent responsible for bovine spongiform encephalopathy (BSE) or “mad cow disease”.

7.3.9.4 Use of Equipment

- a. Perform routine verification of autoclave working condition (refer to Section 8.5).
- b. Do not use laminar flow benches when using cell lines potentially containing infectious agents. Refer to Chapter 5 for more information on the proper use of various containment equipment.
- c. Ensure that you understand the correct operation of a biological safety cabinet (refer to Section 5.2).
- d. Protect all vacuum lines used for aspirations of cultures, with disposable air filters and/or liquid traps. Refer to Section 5.4 for more information.

7.3.9.5 Decontamination

- a. Decontaminate all cell culture wastes by autoclaving or with an appropriate chemical disinfectant (refer to Section 8.4).
- b. Decontaminate all reusable glass and plastic ware immediately after use. Do not allow a laboratory worker / dishwasher to be exposed to contaminated laboratory ware.
- c. Clean up any culture fluid spills immediately with a validated disinfectant (refer to Section 12.3).

7.3.10 Safe Handling of Human & Non-Human Primate (NHP) Derived Cell Lines

Laboratory-associated infections can occur from handling human or primate primary cell cultures, human blood, body fluids or tissues. Examples of potential hazards include:

- HBV, HIV or other blood borne pathogens present in blood or body fluids,
- Agents such as *Mycobacterium tuberculosis* that may be present in human lung tissues (or even non-human primate tissue, especially tissue from wild animals)
- Cells transformed with viral agents, such as SV-40, EBV, or HBV
- Cells carrying viral genomic material
- Tumorigenic human cells present a hazard if self-inoculated
- Risk from adventitious contamination of cell cultures (any mammalian cell culture may provide a support for contaminating adventitious bacteria, fungi, mycoplasma or viruses that cause harm to human health).

Handle all human and primate cells using **Biosafety Level 2** practices and containment. Since most cell lines are not fully characterized, it is wise to regard such cell lines as potentially infectious and handle them at the same biosafety level as a cell line known to carry HIV or Hepatitis B virus. Further HIV route of infection should be considered and Hep B screening and/or vaccination is mandatory for staff and students.

Perform all work in a BSC when handling infectious materials, or when there is a potential of aerosol production. Decontaminate all material by autoclaving or disinfection before discarding.

A Hepatitis B immunization is required for all personnel handling human cell lines.

All established or permanent cultures of human lymphocytes should be handled on the assumption that they harbor the Epstein-Barr virus.

7.3.11 Safe Handling of Recombinant DNA

DNA only gains a biological function by being inserted into a living cell. Hence work with most 'naked' DNA molecules is not generally thought to constitute a safety hazard. Some degree of risk may still exist as such molecules can enter the cells of the operator through breaks in the skin. A greater degree of risk is associated with:

- DNA encoding an oncogene or tumour suppressor gene product
- DNA encoding growth factors, growth factor receptors or other substances that might alter the growth patterns of human cells directly or indirectly.
- Viral DNA or RNA representing complete viral genomes or fragments with the potential to regenerate live virus.

Exercise good laboratory practice when handling isolated DNA molecules ('naked' DNA) through recombinant DNA techniques such as polymerase chain reactions (PCRs), gel electrophoresis, restriction enzymes digestion, sequencing, etc. to prevent skin contact or injection as detailed in the earlier sections.

Full length viral DNA/RNAs are infectious in their own right and are regarded as micro-organisms even when they are not encapsulated or enveloped. This means that if full-length viral DNA were to be combined with DNA from other sources, a genetically-modified organism would have been created. Refer to Appendix 5 of [Singapore Biosafety Guidelines for Research on Genetically Modified Organisms \(GMOs\)](#) by the Genetic Modification Advisory Committee (GMAC) for guidance on working with viral DNA/RNA.

Recombinant DNA also covers all biological entities (cells, organisms, prions, viroids or viruses) that have been genetically modified through recombinant DNA technologies. Evaluate the potential biohazard associated with a particular genetic modification to select the appropriate biosafety level for such work. Refer to Chapters 5, 9 and 11 for detailed guidelines for safe containment, handling and transport of genetically modified organisms used in research.

7.4 WORKING WITH LENTIVIRUS VECTORS

Lentiviral vectors are mostly HIV-based gene delivery vehicles which have the unique characteristic of integrating into the genome of non-dividing cells as well as dividing cells.

7.4.1 Definitions

- a. **Advanced Generation Lentiviral Vectors** include:
 - i. Any third or higher generation lentiviral vector/s (mainly commercially available) or

- ii. Any vector with all of the following safety features:
- Comprises a minimum of 4 plasmids in total which includes all types of plasmids (e.g. packaging, structural, accessory, etc.)
 - HIV genes are split to a minimum of 2 packaging plasmids
 - the *env* gene encoding for the HIV envelope is replaced
 - the *vif*, *vpr*, *vpu* and *nef* genes are either absent or altered to be non-functional
 - the *rev* and *tat* genes are absent, non-functional or expressed from a separate construct; and the *gag* and *pol* genes are split over at least 2 plasmids or there is deletion in the 3'LTR or both.

This definition is provided in the Biological Agents and Toxins List updated by MOH Regulatory Policy Branch (Biosafety Team) on 9 Jan 2012.

- b. **Non-Advanced Generation Lentiviral Vectors** are first and second generation (lower generation) lentiviral vectors or vectors that do not have all of the above mentioned safety features.

Publication from Pauwels K *et al.* (2009) can be referred for more details on the definition of different generations of lentiviral vectors.

7.4.2 Approvals for Lentiviral Work

Refer to **Appendix A.2** under Appendices for the guide to the stepwise procedure for approval for possession and permission to work with lentiviral vectors.

7.4.2.1 Submission of Risk Assessment

Once a decision has been made to use a lentiviral vector system, the first thing that needs to be done is to submit a risk assessment to OSHE for approval by IBC. Even though PIs may be certified under the lab certification scheme, depending on the proposed work it may still require the submission of a risk assessment before starting to use lentiviral vectors.

Please refer to Section 7.4.2.3 for risk assessment submission for non-advanced generation lentiviral vector use.

Please refer to 7.4.2.4 for risk assessment submission for advanced generation lentiviral vector use.

7.4.2.2 Submission of proposal for genetic modification work

Depending on the proposed vector system or the proposed work, genetic modification work with lentiviral vectors may require endorsement by the Genetic Modification Advisory Committee (GMAC), which is a national level committee that reviews and endorses genetic modification work of moderate to high risks. Please refer to [Assessment Flow Chart on Regulatory Requirement for Genetic Modification](#) (OSHE/SH/BS/03) for more information on how to assess the regulatory requirements.

Section 7.4.2.3 below describes how the work with non-advanced generation lentiviral vectors fall under the extent of the [Singapore Biosafety Guidelines for Research on Genetically Modified Organisms \(GMOs\)](#) while Section 7.4.2.4 describes how the work with advanced generation lentiviral vectors fall under these guidelines.

Please refer to GMAC website for information on [Singapore Biosafety Guidelines for Research on Genetically Modified Organisms \(GMOs\)](#) and to download the [Proposal Form for Assessment of Genetic Manipulation Work](#) form.

The GMAC [Proposal Form for Assessment of Genetic Manipulation Work](#) must be submitted to OSHE along with the risk assessment form for approval.

7.4.2.3 Specific requirements for use of non-advanced generation lentiviral vectors

Non-advanced generation lentiviral vectors are regulated under the First Schedule, Part I of the Biological Agents and Toxins act (BATA) by the Ministry of Health (MOH) Regulatory Policy Branch (Biosafety Team). As such IBC approval, GMAC endorsement and MOH approval need to be sought before starting the work.

If a PI wishes to use a non-advanced generation lentiviral vector/s this is the sequence of steps that should be followed for obtaining the necessary approvals / endorsements:

- a. A risk assessment must be submitted to OSHE for review and approval by NUS IBC.

Regardless of whether a PI is certified under the lab certification, a complete risk assessment needs to be submitted when non-advanced generation lentiviral vectors are proposed to be used. For new projects please use the [integrated Online Research Compliance \(iORC\)](#).

For ongoing projects, please contact OSHE biosafety team to find out the format of the risk assessment form to be submitted.

- b. Together with the OSHE risk assessment, submit the [Proposal Form for Assessment of Genetic Manipulation Work](#). OSHE will forward this proposal on behalf of the researcher to GMAC for their review.
- c. After IBC approval, liaise with OSHE in completing the application letter to the Regulatory Policy Branch (Biosafety Team) of MOH. An additional risk assessment form prescribed by MOH Regulatory Policy Branch (Biosafety Team), SOP for working with lentiviral vectors and any other supporting documents need to be prepared at this stage.

(For the template of the application letter and the additional risk assessment form, please refer to **Appendix A.3 and A.4**, respectively).

Once completed, OSHE shall forward the application letter and supporting documents to MOH Regulatory Policy Branch (Biosafety Team) on behalf of the researchers and assist in any communication between MOH and the researchers.

Generally MOH approval would be granted, only after endorsement from GMAC has been given. But to expedite the procedure the researchers are encouraged to do the submissions to GMAC and MOH concurrently.

- d. Once MOH approval is granted the following steps have to be carried out for the registration of the PI's proposed lentiviral facility and personnel with MOH.

- Inspection of the facility by OSHE – this may be done prior to IBC approval if necessary. Please note that facility inspection will be an annual requirement for every calendar year until the proposed work is completed.
- Submission of information related to the users and procurement of the vector to OSHE, who will help in submitting this information to MOH Regulatory Policy Branch (Biosafety Team) for online registration of facility and possession. To submit the personnel details please use the table that can be found as **Appendix A.5**.
- Once the online registration is complete, applying for an import permit if any importation is involved. Please refer to Section 7.4.3 for more details.
- Strict adherence to the safety precautions outlined in Section 7.4.5.
- Submission of the annual report of lentiviral work to MOH via OSHE at the end of each year. Please note that even if work has not commenced after the approval has been granted, the annual report still need to be completed and submitted to MOH no later than January 31 of the next calendar year.

OSHE needs to be notified if any of the following scenarios are to take place:

- Transfer of the lentiviral material to another facility
- Receipt of lentiviral material from another facility
- Failure of receipt of transferred or imported samples/stock
- Disposal of the lentiviral material at the end of the project
- Export
- Near misses
- Accidents
- Laboratory acquired infections arising from the handling of the lentiviral material

If deemed necessary, OSHE shall inform / report these events to MOH on behalf of the researchers.

- e. The risk assessment and GMAC approvals are specific to the lentiviral vector system(s), the gene(s) of interest or the purposes stated in the application. The use of any new non-advanced generation lentiviral construct that involves a different transgene or a different type of alteration other than that already approved by the IBC and GMAC must be communicated to the IBC/OSHE who will advise on the documents that need to be submitted to ensure that appropriate risk assessment has been conducted and approved for the proposed changes. In addition, a new proposal to GMAC needs to be submitted to GMAC through OSHE.

Please note that if, during the course of work, it is decided to directly use lentiviral vectors on animals IBC/OSHE and IACUC approvals shall be sought and GMAC endorsement may also be needed. MOH need to be notified of the animal work.

- f. The lentiviral vector and its derivatives must be destroyed after 3 years or on completion of project depending on whichever comes first. If a PI intends to retain the materials after 3 years, the project will have to be reviewed and reassessed by the NUS-IBC, GMAC and also by MOH per the requirements for non-advanced generation vectors.
- g. Each GMAC endorsement is valid for three years. If the work is not completed by the end of three years the endorsement need to be renewed. Contact OSHE for more details on how this can be done.

7.4.2.4 Specific requirements for advanced generation lentiviral vectors

Advanced generation lentiviral vectors are listed under the fourth schedule of BATA. The following sequence of actions needs to be followed by the researchers when advanced generation lentiviral vectors are to be used.

- a. A risk assessment needs to be submitted to OSHE for first-time users of the advanced generation lentiviral vectors. Please contact OSHE for details.
- b. A [Proposal Form for Assessment of Genetic Modification Work](#) may need to be submitted if the transgene / insert that is used with the advanced-generation lentiviral vector system has one or a combination of the characteristics listed below:
 - Oncogene (expression)
 - Tumor suppressor (knock down)
 - Cell growth regulator
 - Toxin
 - Pathogenic determinant
 - Genetic material that has not been fully characterized derived from a biological agent capable of causing disease in humans, animals or plants
 - Capable of increasing virulence of host or vector

GMAC endorsements are specific to the lentiviral vector system(s), the gene(s) of interest or the purposes stated in the application. The use of any new lentiviral construct that involves a different transgene or a different type of alteration may require another application to GMAC. Refer to the [Singapore Biosafety Guidelines for Research on Genetically Modified Organisms \(GMOs\)](#) or consult OSHE if in doubt.

Please note that each GMAC endorsement is valid for three years. If the work is not completed by the end of three years the endorsement needs to be renewed. Contact OSHE for more details on how this can be done.

- c. Once GMAC endorsement has been granted, apply for import permit if any importation is involved.

Please note that even if the GMAC proposal has been classified as a Category B proposal that only require notification, if importation is involved, the vector can be imported only after GMAC endorsement has been granted. This is because MOH, who is the final authority that issue the import permit, will look for GMAC endorsement before issuing the permit.

7.4.3 Importing Lentiviral Vectors

An import permit is required for the importation of the lentiviral vectors belonging to all generations, when the whole vector system is imported at the same time. The importation of lentiviral vectors can only be done after approval / endorsement from the relevant authority has been sought.

If a local vendor is an authorized dealer for the intended lentiviral vector, the vendor can import the lentiviral vector on behalf of the researcher.

For direct importation from an overseas source, a local forwarding agent or courier company can be engaged to apply for the import permit on behalf of the researchers.

Please note the MOH Product codes assigned to the lentiviral vectors:

- for non-advanced generation lentiviral vectors - [MOHHP3VRETRHUM04](#)
- for advanced generation lentiviral vectors - [MOHHP2VRETRHUM01](#)

This code needs to be supplied to the forwarding agent / courier for application of the import permit.

The import permit is applied for through the [Singapore Customs TradeNet System](#), where the permit granting authority would be the MOH Regulatory Policy Branch (Biosafety Team).

The recipient / consignee must ensure that a correct and valid import permit has been obtained for each shipment of the lentiviral vector and kept on record. A copy can be requested from the local vendor, forwarding agent or courier.

When importation of non-advanced generation lentiviral vectors is involved, all documents related to importation and transfer (import permit, hazmat driver license for the driver who delivered the vector and all other documents) must be filed and kept for reference purposes.

7.4.4 Transport

Non-advanced generation lentiviral vector / constructs can only be transferred to another laboratory approved for the particular non-advanced generation lentiviral work. Notification of the transfer must be made to OSHE prior to the transfer.

The use of public transportation (including taxis) is strictly prohibited as specified in the Biological Agents and Toxins Act, for transportation of advanced generation as well as non-advanced generation lentiviral material.

Please refer to Chapter 9 for transportation requirements for lentiviral vectors.

7.4.5 Safety Precaution

Work with lentiviral vectors should be done in a BSL2 facility with some BSL3 practices, which is known as BSL2+ containment.

In order to mitigate the biological safety risks (see **Appendix A.6**) involved in lentiviral vector work, the following safe practices should be followed. Please note that in an effort to ensure greater safety no distinction has been made between practices for advanced generation and non-advanced generation lentiviral vector work. In other words, the same set of practices is prescribed for all types of lentiviral vector work. But it should be mentioned that some experiments may require higher safety practices than what is described below. This shall be decided jointly by the PI, the Faculty Safety & Health Officer, OSHE, and the NUS IBC.

7.4.6 Training

- a. ***No one is allowed to work with lentiviral materials without prior training*** by the PI / Supervisor who supervise their work.
- b. All personnel who handle recombinant viruses should be provided with on-the-job training covering the biology of the virus, risks with lentiviral work, protective measures to follow and emergency response procedures in the event of an exposure or a spill.
- c. All personnel should demonstrate a good understanding of this SOP as a minimum and any other lab-specific SOP on lentiviral work and demonstrate competency to the satisfaction of the PI/Supervisor in performing the lentiviral work prior to being permitted to work with lentiviral vectors.
- d. PIs/Supervisors are fully responsible to ensure that staff and students under their care are well trained and proficient in the laboratory techniques before they are allowed to work unsupervised.
- e. For all personnel working with non-advanced generation lentiviral work, what kind of training has been provided shall be documented and documentation maintained. Personnel working with advanced generation lentiviral work can also maintain records of the training provided.

7.4.7 Containment Facilities

- a. All lentiviral vector work should be conducted minimally in a BSL2 facility. The lentiviral vectors and their derivatives shall be accessible only by authorized personnel.
- b. Where possible, the work should be conducted in a tissue culture/ viral culture room designated for the lentiviral work. The use of a dedicated set of equipment, such as a biosafety cabinet (BSC), incubators, pipettes etc. for the lentiviral work, is highly recommended.
- c. The designated room and equipment should be labeled with a “biohazard” sign and a label indicating “Lentiviral vector work”. In addition, information on emergency contact personnel and required personal protective equipment should be indicated on the signage. Refer to Section 6.1 on signs and labels.
- d. All procedures involving lentivirus should be conducted within a biological safety cabinet to prevent the risk of aerosol exposure.
- e. Personnel are to follow strict operating procedures when working in the BSC. Please refer to Section 5.2 on biological safety cabinets.

7.4.8 Safe Practices

- a. Production of a lentiviral vector and its derivatives should be limited to a laboratory-scale of not more than 100 ml.
- b. Centrifugation must be performed in screw capped tubes (including microfuge tubes) and with the use of aerosol containment devices (e.g. sealed canisters, aerosol-containing safety cups, buckets or rotors, etc.). The tubes shall not be over-filled and the outer surface shall be decontaminated with an efficacious disinfectant before removing from the BSC. It is required that aerosol resistant centrifuge cups should be opened only in the BSC after centrifugation. Allow aerosols to settle for one to 5 minutes before opening the centrifuge. For further details on safe use of centrifuges please refer to Section 5.6 on centrifuges.
- c. It is recommended to avoid / minimize the use of sharps especially if the transgene in the vector construct has oncogenic properties. All sharps, if any, should be disposed off immediately into a sharps container within the BSC. It is advisable to use plastic disposable transfer pipettes rather than glass pipettes. The use of safety engineered needles, disposable scalpels and

safety engineered scalpels is highly recommended. Please refer to Section 7.5 on safe handling of sharps.

- d. Culture vessels containing lentiviral material should be placed in a secondary container when being transferred from the biological safety cabinet to the incubator to reduce the risk in case of a spill. The vessels should be kept in a designated incubator, labelled “Lentiviral Work” whenever possible. Care must be taken to avoid contamination of the incubator door handle.
- e. There should be controlled access to lentiviral stock produced. For storage of lentiviral materials (e.g. culture supernatant) in freezers / liquid nitrogen tanks, the containers used for storing lentiviral materials must be robust and leak-proof. The containers must have the biohazard sign and labelled with description of the lentiviral material and name of personnel. It is recommended to always keep in one designated compartment of the freezer / liquid nitrogen tanks for storage of lentiviral materials.
- f. An inventory of the lentiviral vector or constructs that are stored within the lab should be maintained. This is a strict requirement for the non-advanced generation lentiviral vectors.
- g. It is recommended to remove gloves and wash your hands thoroughly with soap immediately after handling lentiviral material (or animals / animal / human cell lines associated with lentiviral material) and also before leaving the laboratory area.

7.4.9 Personal Protective Equipment (PPE)

- a. The minimum PPE required when handling lentiviral materials include: Gloves, laboratory coats, covered shoes and face/ eye protection. Respirators should be worn when appropriate (such as when cleaning up spills outside the containment of a biosafety cabinet) and personnel should be fit tested for these respirators. Lab personnel should be trained to use the PPE appropriately.
- b. Gloves must be worn during all tissue culture manipulations and when handling lentiviral materials. It is advisable to use double gloves as micro-holes may be present. Gloves should be examined for obvious holes prior to use. The outer gloves should be changed at regular intervals and when contaminated. It is recommended to ensure that no skin is exposed between the gloves and the lab coat and to this end lab coats with elasticized cuffs can be used, over which outer gloves can be worn.
- c. Avoid touching any part of your body (skin, face, eyes, mouth, etc.) with your gloved hands as infectious materials deposited on your gloves can come into contact with body areas as a result.
- d. When working within the BSC, it is recommended to treat the outer gloves as contaminated – remove them and replace with new and clean gloves before touching objects outside the biological safety cabinet (e.g. the microscope, the centrifuge, or the incubator).

7.4.10 Decontamination / Waste Disposal

- a. All materials that have come in contact with lentiviral materials must be decontaminated via chemical means / autoclaved before disposal.
- b. Gloves and lab coat should be worn when performing decontamination procedures.
- c. On completion of work, the outer surface of all materials in the BSC should be decontaminated and then removed. The BSC should be thoroughly wiped with an appropriate efficacious disinfectant.
- d. If there is any reason to suspect that equipment (e.g. incubators, microscopes, and centrifuges, etc.) used for lentiviral work has been contaminated, it must be decontaminated immediately

following use. The manufacturers' recommendations for decontamination of the equipment should be followed.

- e. Used paper towels and gloves should be placed in a biohazard bag and subsequently autoclaved prior to disposal.
- f. The biohazard wastes containing lentivirus-contaminated dishes, filters, plastic ware, gloves etc. should be collected in a biohazard bag inside the BSC and the outside wiped with an appropriate efficacious disinfectant. Subsequent to completion of work, the bag must be sealed within the BSC and transferred to a second bag and taken to the designated autoclave for autoclaving / disposed as per building / institute waste disposal policy.
- g. Pipettes used for tissue culture work should be disposed in an appropriate way. Please consult OSHE or the faculty safety officer to decide on a method that best suits your needs. The use of glass Pasteur pipettes is strongly discouraged.
- h. All liquid wastes such as tissue culture waste and media containing viral material should be decontaminated by autoclaving or with the use of an appropriate chemical disinfectant in accordance with the manufacturer's recommendations. If a vacuum pump for the BSC is used, it must be fitted with a hydrophobic vacuum line filter meeting HEPA specifications (filtering at least 99.97% of particles greater than 0.3 μm).
- i. Liquid spills on any surfaces should be immediately disinfected with freshly prepared bleach or an efficacious disinfectant. It is important to ensure that the final concentration of the disinfectant after adding into the spill material would be the effective concentration (for example if 10% bleach is the effective concentration then the initial concentration of the bleach should be higher to compensate for any dilution that happens after adding the bleach into the spilled liquid/material. The appropriate contact time should be ensured. If potentially corrosive disinfectants such as bleach are used on stainless steel surfaces it is important to rinse the disinfectant off using distilled water.

7.4.11 Precautions Related to Animal work

- a. In general, work with lentiviral vectors, including the administration into animals should be conducted using BSL2 practices and facilities, and should be done inside the containment of a biological safety cabinet, whenever possible.
- b. ABSL-2 housing should be used for the infected animals, however, due to the controls built into most of the advanced generation lentiviral vectors and because many animals cannot support the replication of HIV-1, the housing controls may be downgraded to ABSL1 after 72 hours post-infection, subject to NUS IBC / OSHE approval. OSHE and CM should be consulted to see whether disposable cages may be needed to house the animals post-infection.
- c. Animals engrafted with human cells, humanized animals or animal hosts that are permissive for HIV-1 replication constitute special cases in light of their potential to support replication of infectious HIV-1. Use of lentiviral vectors in these animals may require ABSL2 housing throughout the whole post-infection duration.
- d. Animal carcasses must be disposed of according to the animal facility protocol.
- e. It is recommended that only needle locking syringes or disposable syringe-needle units should be used for animal injections.
- f. Animal care and veterinary staff must be informed if any specific handling procedures are needed or if any other special conditions apply. Door warning signs and cage signage should reflect the vector being used, PI's contact details, as well as the biohazard symbol.

7.4.12 First Aid

- a. Following a splash or accidental contact of eyes, nose or mouth with material potentially contaminated with lentivirus, the contact surface must be immediately flushed at an eyewash station for 15 minutes. Saline is recommended if it is available, as water can be irritating to the eyes for this length of time. Gloves must be removed and hands washed before using them to keep the eyes open
- b. A wound or injured skin potentially contaminated with lentiviral material should be immediately washed with soap and kept under running water for 15 minutes.
- c. Medical attention should be sought if exposure to lentiviral vectors occurs (see Chapter 12 on Exposure Management).
- d. Medical physician may recommend post-exposure anti-retroviral chemoprophylaxis.

7.4.13 Biological Spill Response Procedure

Please refer to Section 12.3 on biological spill response for detailed procedures on handling biological spills.

7.4.14 Medical Surveillance

Medical surveillance and examination for non-advanced generation lentiviral work is required, and that post-exposure treatment shall be determined by the attending OH Physician upon exposure to such agents.

7.4.15 Accident / Incident Reporting

All accidents and incidents must be reported within 24 hours to the PI, the Faculty Safety and Health Officer and to OSHE via the online [Accident / Incident Reporting System \(AIRS\)](#). Please refer to Section 12.4 for more details.

7.4.16 Records

- a. Template for application letter to MOH Regulatory Policy Branch (Biosafety Team) – to seek approval for possession and use of non-advanced generation lentiviral vectors (**Appendix A.3**).
- b. Additional risk assessment form (**Appendix A.4**).
- c. [Lentivirus Annual Report Template](#).

7.5 SAFE HANDLING OF SHARPS

Sharp(s) - Any object used or encountered in a laboratory setting that can puncture or penetrate the skin or any other part of the body and result in an exposure incident. Examples of laboratory items that fall under this category include but are not limited to, needle devices (hypodermic needles, suture needles, etc.), scalpels, lancets, Pasteur pipettes, broken glass, and broken capillary tubes and disposable syringes.

Sharps injury - Any injury caused by a sharp, including, but not limited to, cuts, abrasions, or needle sticks.

There are two aspects to dealing with sharps: using them and throwing them away. Both can be risky and require special care.

Use the hierarchy of controls concept to prioritize prevention interventions:

- The first priority is to eliminate and reduce the use of needles and other sharps where possible.
- Second is to substitute with safer alternatives.
- Next is to isolate the hazard, thereby protecting an otherwise exposed sharp, through the use of an engineering control.
- When these strategies are not available or do not provide total protection, consider work-practice controls and personal protective equipment.

7.5.1 Elimination / Substitution of Sharps

The best way to avoid a sharps injury is to avoid using sharps, e.g. needles and syringes should never be used as a substitute for pipettes!

Avoid using needles and syringes whenever possible because the majority of laboratory biohazard injuries are due to needlestick injuries. The use of needles and syringes should be restricted to procedures for which there is no alternative.

Where possible, find alternatives to using needles such as:

- Using alternate routes for inoculation, medication delivery or vaccination to animals e.g. blunt cannulae or IV delivery systems that do not require needle access.
- Reviewing specimen collection systems to identify opportunities to consolidate and eliminate unnecessary punctures.

Substitute sharps with plastic where possible. For example:

- Disposable transfer pipettes may be a good replacement for glass Pasteur pipettes.
- Replace lab glassware with plastic ware wherever possible. It can eliminate broken glass problems.

Use round-tipped scalpel blades instead of sharp-tipped blades

7.5.2 Engineering Controls

Use devices with engineered sharps injury prevention features e.g. safety-engineered blood collection needles. These devices have a built-in physical attribute such as blunting, needle withdrawal, automatic needle-resheathing or other physical mechanism which effectively reduces the risk of an exposure incident.

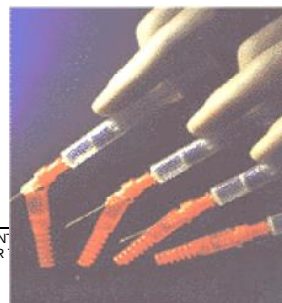
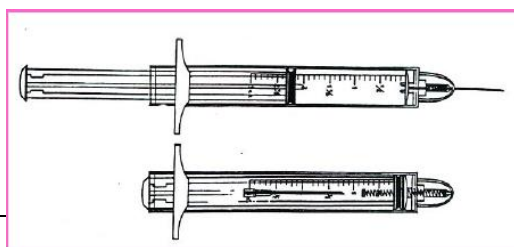


Figure 7.3 Safety-engineered needles

Use a sharps disposal container for the disposal of sharps and place it close to where sharps are being used. The container should be durable, closable, leak resistant and puncture resistant. It should be clearly visible, with appropriate warning signals, and be easily accessible to lab personnel who use / dispose of sharp devices. These are typically available commercially in different sizes as yellow plastic containers with a biohazard symbol. Use an appropriate sized container to minimize protruding sharps.



Figure 7.4 Example of a sharps bin

7.5.3 Work-Practice Controls

When sharps must be used, the following procedures are recommended:

a. General controls

Use instruments rather than bare hands to handle sharps. For example, use forceps to load or unload needles and scalpels; use tongs to pick up broken glass.

Give verbal announcements when passing sharps.

Do not leave unprotected sharps (razor blades, scalpel tips, etc.) unattended on bench tops. Contain the sharp items in a tray or a suitable container.

Be careful when cleaning up after experimental procedures that require the use of sharps as sharp items may have become hidden in the garbage.

Lab glassware that are chipped or cracked should be discarded.

b. Syringes and Needles

As the majority of laboratory biohazard injuries are due to hypodermic needles, special attention must be paid to the use and disposal of needles to minimize the possibility of exposure via accidental autoinoculation.

To attach a needle to a syringe, insert the small end of the syringe into the hub of the **capped** needle.

To remove a needle cap, hold the syringe with one hand and use the other hand to grasp and push the needle cap toward the syringe while rotating the cap just slightly (about 1/4 turn) to break the seal. Do not try to pull the cap off the needle as you may inadvertently stick yourself when the cap comes off suddenly.

Never leave an uncovered needle on the counter. Always rest the needle in its cap while waiting to use the assembled needle and syringe or in between steps of a procedure. It is not necessary to place the cap securely onto the needle at this point.

Do not walk around the lab with an uncapped needle or syringe and needle

Do not bend, break, or otherwise manipulate needles BY HAND.

Never recap needles as an accidental puncture may occur. If it is absolutely necessary, recap use a cap-holding device or a pair of forceps or a one-handed technique to scoop the cap up.

Use disposable needle locking syringe units whenever possible. Do not remove needles from syringes. Throw away the entire syringe-needle combination.

When using syringes and needles with biohazardous or potentially infectious agents:

- Work in a biological safety cabinet and avoid quick and unnecessary movements of the hand holding the syringe.
- Wear surgical or other type of rubber gloves
- Use needle-locking (Luer-Lok type) syringes.
- Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.
- Expel air, liquid and bubbles from the syringe vertically into a cotton/gauze pad moistened with disinfectant.
- Do not use a syringe to mix infectious fluid forcefully so as to prevent aerosol formation.
- Do not contaminate the needle hub when filling the syringe in order to avoid transfer of infectious material to fingers.
- Wrap the needle and stopper in a cotton pad moistened with disinfectant when removing a needle from a rubber-stoppered bottle.
- Use a separate pan of disinfectant for reusable syringes and needles. Do not place them in pans containing pipettes or other glassware in order to eliminate sorting later.

7.5.4 Sharps Disposal

Proper disposal of sharps is essential for protecting cleaners / waste disposal workers, and the general public from being injured by discarded sharps.

Never discard sharps into regular trash bins or biological waste.

Dispose of all sharps into puncture resistant containers that do not allow the whole hand to go in.

Never try to recover sharp material after it has been disposed off into the sharp container.

When disposing needles and syringes, do not recap the needle nor remove the needle from the disposable syringe. Place the **entire** needle-syringe unit directly into the sharps disposal container.

All contaminated sharps are to be treated as infectious and disposed of only through licensed biohazardous waste collectors.

Contaminated broken test tubes or other small items of broken glassware should be placed directly into Sharps containers. Larger pieces of **clean** broken glassware (e.g. beakers, flasks, test tubes, etc. free from biohazardous, radioactive or chemical contamination) can be discarded into the standard broken glassware boxes. If the glassware is contaminated disinfect it before disposal.

Do not place sharps containers on the floor of the lab at all times

Keep sharps containers covered at all times except when sharps are being deposited into the container.

Do not overfill sharp containers beyond the recommended fill line or beyond 3/4 full.

Filled sharps containers should be sealed, labeled as 'SHARPS' and the biohazard symbol before disposal by licensed biohazardous waste collectors for incineration.

Refer to Section 8.4 for more details on the waste disposal procedure.

7.5.5 Sharps / Needlestick Injury

In the event of a sharps or needlestick injury, encourage bleeding, wash the punctured wound with soap and water and apply an appropriate skin disinfectant.

Inform the principal investigator or laboratory supervisor about the cause of the wound and organisms involved if any.

In the event of exposure to biological materials/infectious agents resulting in possible infection, disease or illness, please call at the University Health Center (UHC) or Occupational Health Clinic for a medical assessment or proceed to the Accident & Emergency Units of Hospitals after office hours.

University Health Centre (UHC)

20 Lower Kent Ridge Road
University Health Centre, Level 1
Singapore 119080
Tel: (65) 6776 1631
E-mail: uhc_health@nus.edu.sg

Occupational Health Clinic

Office of Safety, Health & Environment (OSHE)
University Health Centre, Basement
20 Lower Kent Ridge Road
Singapore 119080
General enquiries: (65) 6516 7333 / 6601 1781

Submit a report to OSHE via the online [NUS Accident / Incident Reporting System \(AIRS\)](#).

SAFE HANDLING OF LABORATORY EQUIPMENT

There are a number of equipment in the lab that require extra care in their use. It is essential to have adequate training in the use of all equipment before attempting to use it. Do not use any equipment if you have not had training on or are unsure of their use. Commonly used equipment covered in this manual include:

- Autoclaves – see Section 8.5
- Biological Safety Cabinet – see Section 5.2
- Blending, Mixing, Sonicating and Cell Disruption Equipment – see Section 7.7
- Bunsen Burners – see Section 7.8
- Centrifuges – see Section 5.6
- Chemical Fume Hood – see Section 5.5
- Electrophoresis equipment – see Section 7.9
- Flow Cytometer – see Section 7.6
- Hot plates, drying ovens, and other heating devices – see Section 7.10
- Lyophilizer / Freeze dryers – see Section 7.11
- Microscopes – see Section 7.12
- Microtomes / Cryostats – see Section 7.13

7.6 FLOW CYTOMETER

Advances in cell biology have increased the need for live, infectious cell sorting and analysis of cell cultures and experiments involving molecular genetics. These instruments utilize either a jet-in-air flow cytometer or a cell sorter that combines a flow cell with jet-in-air sorting technology.

Droplets are created from a liquid stream carrying the cells through a nozzle vibrating at a high frequency and are passed between high-voltage plates. Operator selected parameters electrostatically charge and deflect the droplets containing the cells of interest into containers. Droplet size is dependent on the instrument's operating pressure, size of the nozzle orifice and its vibration frequency. All sorters produce micro droplets during normal operation and even larger amounts of secondary aerosols are produced when a clog in the nozzle causes deflection of the fluid stream that hits a hard surface such as a waste collector or the walls of the collection chamber.

If these aerosols are not contained, they pose a potential health risk to sorter operators, and the environment. Although there is no documented case of a laboratory acquired infection (LAI) in a flow cytometer facility, 82% of LAIs are of unknown origin and aerosol generating procedures are the main suspects for these. Infections in the laboratory may occur even if the agent is not normally transmitted via aerosols. There are documented cases of hepatitis B, normally transmitted through the percutaneous route, being aerosolized and causing infection. Although fixing of the cells may reduce the risk from pathogens, the efficacy of fixation must be verified.

Recent studies by the National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health which used an aerodynamic particle size to determine the concentration and aerodynamic diameter (AD) of aerosols produced by a FACS Aria II cell sorter under various conditions (Holmes, 2011). It was demonstrated that “even with an operational aerosol management system (AMS), aerosols created by a fail mode were not efficiently evacuated from the sort chamber. It is therefore possible that following a nozzle obstruction, on opening the sort chamber door, the operator

may be exposed to aerosols that are contained with the sort chamber.” Therefore, many institutions require operators to wear respiratory protection when sorting unfixed human samples or samples containing human pathogens, using BSL3 practices in a BSL2 facility.

Samples may not only contain bloodborne pathogens associated with human cells, but may also be labeled with toxic and/or carcinogenic dyes which can pose additional risks. Unfixed specimens containing potentially infectious agents should be handled at BSL2, BSL2+ (BSL2 facility with BSL3 practices) or BSL3 (facility and practices), depending upon the risk assessment.

Engineering controls, training, and Personal Protective Equipment (PPE) requirements for the use of a stream-in-air cell sorter are dependent on the facility, type of sample, species of cells, and pathogens.

Flow cytometers which are analyzers do not pose the same potential for aerosol production as the cell sorters as they operate at lower pressures (<30 psi). The following recommendations primarily apply to cell sorters. However, similar precautions may be applicable to cell analyzers too, especially when handling potentially infectious agents and unfixed samples.

7.6.1 Risk Assessment

- a. Risk assessment must be conducted prior to cell sorting procedures. Refer to **Table 7.1** for roles and responsibilities of Principal Investigators, Cell Sorting Facility Manager and Operator.
- b. Refer to **Table 7.2** for general guidelines for determining the BSL and general procedures for cell sorting.
- c. Refer to **Table 7.3** for examples of specific agents and recommended Biosafety levels.
- d. Comprehensive risk assessments are often completed in collaboration with specialists, subject matter experts, the biosafety specialist, and the Institutional Biosafety Committee (IBC). Risk assessments should identify risks associated with the sort. Most risks can be determined by the risk groups with reference to [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\), 5th edition](#).

7.6.2 Engineering Controls

- a. Whenever possible, the cell sorter should be placed in a biological safety cabinet, and should be located in a dedicated laboratory room with a BSL2 or higher designation.
- b. The room containing the cell sorter should be dedicated to that instrument so that personnel not involved with the cell sorter are not exposed to potential aerosols. This is especially important if the cell sorter is not in a biological safety cabinet.
- c. The room should have negative air flow in relation to the surrounding areas i.e. air flows from outside the room into the room and into the exhaust system (one pass air). If this is not possible, it is even more crucial that aerosols be contained and filtered.
- d. Access to the room/facility should be restricted to authorized personnel only, and should be operated by trained personnel deemed competent to run the equipment.

7.6.3 Aerosol Management System (AMS)

- a. Cell sorters are equipped with aerosol management systems (AMS). The AMS is designed to evacuate the sort collection area and sort chamber of the cytometer. It transports aerosols through a HEPA or ULPA filter, and then exhausts them to the room.
- b. The AMS must be validated at regular and pre-determined intervals.

- Validation of the AMS is the responsibility of the operator or manager of the cell sorting facility.
 - Tolerance of aerosol escape is zero particles when the AMS is active and the sort chamber door is closed.
 - Validation is conducted according to manufacturer recommendations.
 - Validation is documented; records are kept in the area of the cell sorter and are readily available.
- c. The filter on the AMS must be changed at a minimum of every 6 months.
 - PPE, including a respirator, is worn during filter changes.
 - Used filters are discarded as biohazardous waste.
 - d. The AMS is operated according to manufacturer's guidelines during the sort.

7.6.4 Administrative Controls

- a. The operator must be vaccinated against hepatitis B virus if unfixed human samples are to be handled and sorted.
- b. If available, other vaccinations against infectious agents are recommended if the samples contain other specific infectious organisms, e.g. influenza virus, *Bordetella pertussis*, etc.
- c. Validation on aerosol containment must be performed periodically by the Cell Sorting Facility Manager, such as Glo-Germ assay or equivalent published method of testing the containment and aerosol management system. The Glo-Germ method uses a suspension of highly fluorescent particles with an approximate size range from 1 to 10 µm, simulating a biological sample during the test sort. Aerosol containment is measured by placing microscope slides around the cell sorter where aerosols are produced and examining the slides under a fluorescent microscope for the presence of Glo-Germ particles.
- d. Equipment like cell sorters, cell analyzers and flow cytometers that have Class 3B or Class 4 lasers embedded inside must have an N2 license issued by Center for Radiation Protection and Nuclear Science. Contact your safety officer for assistance in application of this license to the regulatory authorities. Also refer to NUS Laser Safety Manual for further details. The users of these equipment do not need to possess an N3 licence.

7.6.5 Personal Protective Equipment

- a. PPE requirements depend on the BSL designation of the laboratory in which the cell sorter is located and the samples to be run on the equipment. BSL2 PPE is the minimum standard.
- b. If the risk assessment of the unfixed infectious agent being sorted requires **BSL3 practices (BSL2+)**, they would include:
 - Disposable, wrap-around, solid-front, long-sleeved laboratory gown made of fluid resistant material.
 - Double gloves, outer gloves should be changed when torn or contaminated.
 - Operators and other personnel in the room with the cell sorter should wear a respirator during the sorting process and for 30 minutes after the end of sort/machine shut down.
 - i. Respirators should be NIOSH-approved filtering respirators, such as N-95, or equivalent.
 - ii. Alternatively, a powered air purifying respirator (PAPR) may be worn, especially when sorting infectious agents requiring BSL3 practices.
 - iii. Personnel wearing respirators must be enrolled in [NUS Respiratory Protection Programme](#). This includes annual fit-testing, medical clearance exam, and training.

7.6.6 Sample Preparation

- a. Unfixed, potentially infectious samples (BSL2) should be prepared in a biological safety cabinet.
- b. Centrifugation of specimens should be performed in sealed vessels with safety cups.
- c. Avoid the use of needles, glass pipettes, glass transfer pipettes or glass containers or tubes.
- d. A clogged sort nozzle or air in the fluidic system can drastically increase aerosol formation. Reduce the formation of cell aggregates and clumping by following these guidelines:
 - Centrifuge 5-10 minutes ~ 300 x *g* to pellet cells
 - Avoid using thawed samples directly for sorting
 - Keep sort samples at an intermediate temperature (i.e. 15°C) rather than 4°C.
 - Filter samples immediately prior to sorting (perform this procedure in a biological safety cabinet).

7.6.7 Cell Sorting Procedures

- a. Surfaces in the cell sorting facility are kept clean and clear of clutter.
- b. The laboratory door, collection chamber door, and sort chamber door remain closed during cell sorting procedures. **NEVER OPEN THE SORTING CHAMBER WHILE CELLS ARE BEING SORTED.**
- c. If the cell sorter is located inside a biological safety cabinet, sash doors are lowered to the appropriate level according to manufacturer's recommendations, and the hood is operational during procedures.
- d. A spare nozzle and/or O-ring are available in the event of a clog.
- e. Sample tubes are filled with as much sample as possible, but not higher than ¼" from the top of the tube, to minimize loading and unloading of samples.
- f. When changing tubes, wait for 60 seconds before opening the sample chamber door.
- g. Following the sort, tubes are wiped with a 70% alcohol, bleach or an appropriate disinfectant as determined by the risk assessment, as the exterior of the tube may be potentially contaminated.
- h. In the event of a nozzle obstruction:
 - Turn off the system if it has not already shut down automatically. The air evacuation rate on the AMS unit is increased to 100% to clear the clog.
 - Replace the nozzle if necessary.
 - Do not remove the collection tubes from the sort collection chamber until the sample acquisition has been stopped for at least 60 seconds.

7.6.8 Transporting of Samples

- a. Samples are transported in an appropriately labeled leak-proof, secondary container.
- b. If the sample contains biohazardous material, the container must be affixed with a biohazardous material label.
- c. Samples are contained in screw-top tubes, capped tightly.

7.6.9 Disinfection of Equipment

- a. The choice of disinfectant depends on the agent in use, the species of the sample, and the potential for exposure to lab personnel.
- b. A broad-spectrum disinfectant is preferred where agent use is varied.
- c. The instrument is decontaminated with an appropriate disinfectant. A bleach solution or a disinfectant recommended by the manufacturer may be used to disinfect sample lines. This is followed by a sterile water rinse and flush to remove any disinfectant residue.

7.6.10 Exiting Cell Sorting Facility

- a. The AMS is turned off; vacuum gauge is verified at zero.
- b. Surfaces in the cell sorting facility should be disinfected after completion of the sort.
- c. Disinfectant is added to the waste collection vessel, allowed to inactivate the biohazardous waste fluid for 10-20 minutes, and then disposed of down the drain with copious amounts of tap water.
- d. All disposable PPE is removed and placed into a biohazardous waste container.
- e. Hands are washed thoroughly with soap and water.

7.6.11 Emergency Procedures

- a. Refer to department-specific policies and procedures.
- b. In the event of a spill, follow biological spill clean-up procedures according to the risk group level of the infectious agent.
- c. In the event of a serious injury or emergency, contact NUS Campus Security at **6874 1616** immediately.
- d. Report any injuries / exposures to OSHE within 24 hours via [NUS Accident / Incident Reporting System \(AIRS\)](#).

Table 7.1 Roles and Responsibilities of Principal Investigator, Cell Sorting Facility Manager and Operator

Role	Responsibilities
Principal Investigator	Completes a risk assessment & SOP for cell sorting that considers the following hazards : • types of cells (animal or human) • infectious agents introduced in samples, and corresponding risk group and BSL • mode of transmission of infectious agent • fixed or unfixed samples • location of sorting facility to be used • procedure for transporting cells from laboratory to sorting facility • any special disposal, disinfection issues, or procedures Ensures training for laboratory personnel Communicates to Cell Sorting Facility Manager of the hazards and any special procedures required
Cell Sorting Facility Manager	Reviews the risk assessment conducted by the Principal Investigator Ensures that adequate risk mitigation has been considered by the Operator Provides necessary PPE for the laboratory personnel operating the cell sorter Ensures that all procedures for safe use of cell sorter are followed Performs periodic validation testing of the aerosol containment system

Cell Sorting Operator	Understands the hazards and risk mitigation outlined in the risk assessment which has been reviewed/approved by Cell Sorting Facility Manager Completes appropriate training, if necessary Follow all safe practices and procedures outlined in the SOP, including wearing of the PPE specified Appropriate disinfectant is available and all appropriate surfaces are disinfected, based on the containment testing
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Table 7.2 Biosafety Level Determination for Cell Sorting

CONTROLS	BSL2	BSL2+ (during sorting operations)	BSL3
Risk Assessment Condition	Uninfected non-primate	Non-infectious human/NHP cells	Infectious samples with high risk assessment
		Infectious but with low risk assessment	All samples containing known aerosol pathogens
Example Sample Type or Agents	• Normal murine cells • 3rd gen Lentivirus (non-human cells)	• Normal human or NHP blood • Human or NHP cell lines e.g. Influenza A	Example agents include: • Mycobacterium • Tuberculosis • Monkey pox
Aerosol Management System Validated	Periodically (monthly or with filter change)	Periodically (monthly or with filter change)	Before Every Sort
AM System	Required	Required	Required
HEPA filter on AMS	Changed every 6 months, depending upon type of samples run	Changed every 6 months, depending upon type of samples run	Changed every 6 months, depending upon type of samples run
N-95 Respirator	Optional	Required	Required
PAPR	Optional	Optional	Optional (as a replacement for N-95)
Eye Protection	Face shield or safety goggles	Face shield or safety goggles	Face shield or safety goggles (not needed with PAPR)
Lab Coat /Gown	Disposable, wrap around gown with knit cuffs	Disposable, wrap around fluid resistant gown with knit cuffs	Fluid-resistant coveralls
Separate Room and Environmental Controls	Optional	Required or limited access to room	Required

Table 7.3 Biosafety Level Determinations

This list represents biosafety level determination for cell sorting of specific agents. However, final determination of the biosafety level is dependent upon the risk assessment conducted in collaboration with the Biosafety Office and other subject matter experts.			
AGENTS BEING SORTED	RECOMMENDED BIOSAFETY LEVEL	RESTRICTIONS OR COMMENTS	MSDS LINK^c
Hepatitis C	BSL2+ ^a		Hepatitis C
Influenza A	BSL2+	Influenza (seasonal) vaccine recommended	Influenza A
SIV, SHIV	BSL2+		
LCMV	BSL2+ or BSL3	Ensure that HVAC system does not exhaust near vivarium housing mice; BSL dependent upon strain; pregnant women should consult Occupational Health Services or their personal physician prior to performing a procedure with this agent.	LCMV
Malaria / dengue	BSL2+ ^b		
Listeria	BSL2+	Pregnant women should consult Occupational Health Services or their personal physician prior to performing a procedure with this agent.	Listeria
Vaccinia	BSL2+	vaccine recommended	Vaccinia
H1N1	BSL2+ or BSL3	H1N1 vaccine recommended	
HIV	BSL2+ or BSL3		HIV
TB, Mycobacterium tuberculosis	BSL3		Mycobacterium tuberculosis
^a BSL2+ or “BSL2 enhanced” means a BSL2 facility with some BSL3 practices, e.g. use of respiratory protection ^b Respirator optional (mucous membrane protection required) for this agent except where the sample also contains human/NHP blood cells or fluids ^c Public Health Agency of Canada (http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php)			

7.7 BLENDING, MIXING, SONICATING AND CELL DISRUPTION EQUIPMENT

Devices such as blenders, tissue grinders, magnetic mixers, stirrers, sonic cleaning devices, ultrasonic cell disintegrators, and shakers can generate hazardous aerosols. Operate blending, cell disruption, and grinding equipment in a biological safety cabinet when used with infected tissues or materials and any material of human origin.

Ensure that caps, vessels and gaskets for such equipment are in good condition and caps are well-fitting. Aerosols containing infectious materials may escape from between the cap and the vessel as a result of pressure build up in the vessel during operation of the equipment.

Use plastic, in particular, polytetrafluoroethylene (PTFE) vessels in place of glass as glass may break, releasing infectious material and possibly wounding the operator.

7.7.1 Sonicators

- a. Sonicators are high-frequency sound generators used to disrupt cells or shear nucleic acids. The two major hazards associated with sonicators are hearing damage caused by high frequency sound and generation of aerosols from the sonication process.
- b. Wear hearing protection while using sonicators. Do not sonicate in a room containing people not wearing ear protection.
- c. Shut doors of the room where sonication is taking place and place a warning sign on the door.
- d. Where possible, have the sonicator located in a "sound-proof" cabinet during sonication.

7.7.2 Blenders

- a. Do not use domestic (kitchen) blenders. Use safety blenders designed to prevent aerosol formation and leakage from the bottom of the blender bowl. Such blenders can also withstand sterilization by autoclaving.
- b. Inspect the rotor for leakage prior to operation.
- c. If the blender is used with infectious material place a towel moistened with an appropriate disinfectant over the top of the blender.
- d. Sterilize the device and residual contents promptly after use.
- e. Avoid using glass blender bowls with infectious material because of the potential for breakage.
- f. After blending, allow the contents to settle for a few minutes before opening the blender bowl.

7.7.3 Tissue Grinders

When using glass grinders, hold it in an absorbent material in a gloved hand. Use the safer alternative of plastic (PTFE) grinders if possible.

7.8 BUNSEN BURNERS

- a. Never leave an open flame unattended.
- b. It is extremely dangerous to have open flames around flammable materials, such as ethanol, which are often found in biological laboratories.
- c. Place the Bunsen burner away from combustible materials, papers or chemicals.

- d. Regularly check the hose for cracks, holes or any other defect and ensure that the hose fits securely on the gas valve and the Bunsen burner. Replace all hoses found to have a defect before using.
- e. If you smell gas, turn off the gas valve and wait until the gas has fully dissipated before relighting the burner.
- f. ***Never use a Bunsen burner inside a biological safety cabinet!*** The heat generated within the cabinet creates airflow turbulence that may compromise sterility and worker protection. A biological safety cabinet recirculates air within the interior and explosive vapours may build up inside the cabinet. The heat buildup may also damage the HEPA filters.
- g. You may consider using burners with safety features such as flame monitor, alarm displays, regulated timer, automatic gas cut-off, etc.

7.9 ELECTROPHORESIS EQUIPMENT

- a. Electrophoresis equipment used for agarose gel running, DNA sequencing, etc., presents electrical, chemical and/or radiological hazards.
- b. Most electrophoresis units use very high voltage (approximately 2000 volts) and potentially hazardous current (80 milliamps or more). This high power output has the potential to cause an electrical shock if not properly handled.
- c. Inspect electrophoresis units and their power supplies routinely to ensure that they are working properly. Inspect buffer tanks for cracks or leaks, exposed connectors, and power supplies for signs of deterioration (e.g. exposed wires, damaged leads, etc.)
- d. Place “Danger—High Voltage” warning signs in place on the power supply and buffer tanks.
- e. Practice good housekeeping when locating, or working around or near an electrophoresis unit. Avoid placing the units on grounding points and conductors (e.g. sinks and other water sources or other metal surfaces). Place the equipment where it will not be easy to knock over or trip on. Always maintain adequate clearance around the apparatus.
- f. In the event of spills or leaks of electrophoresis buffer, stop the run and clean up the bench top immediately.
- g. Do not permit leads to dangle below the laboratory bench.
- h. Whenever possible, set the power supply on a shelf above the gel tank.
- i. Turn the power supply off before disconnecting both leads from the power supply.
- j. As with all research, ensure that you are familiar with any associated chemical and radiological hazards and the control measures and precautions required when working with them.

7.10 HOT PLATES, DRYING OVENS, AND OTHER HEATING DEVICES

Burns and other injuries can occur when heating devices such as ovens, hotplates and heating mantles are not used properly.

Never heat a sealed container regardless of the type of heating device used.

Always use the device as intended, especially when used with or near flammable or combustible solvents or materials. Heating devices must be rated / approved for the use and the environment in which it will be used (i.e. flammable or explosive atmospheres, in the presence of combustible dust, etc.)

Use thermal gloves or tongs to remove items from their heating devices.

Use protective eyewear when using ovens, hot plates, or other heating devices.

7.10.1 Drying Ovens

- a. Locate ovens away from potential fuel sources (i.e. common combustible materials like organic solvent storage areas, cardboard and paper, etc.) due to their spark potential.
- b. Due to possible formation of explosive mixtures by volatile substances and the air inside an oven, do not use domestic household ovens in the lab. Unlike laboratory ovens, their heating elements (which may become extremely hot) and their temperature controls (which may produce sparks) are not physically separated from their interior atmospheres.
- c. Drying ovens should not be used to dry glassware that has been rinsed with organic solvents.
- d. Do not use mercury thermometers to monitor oven temperatures. Accidental breakage of the thermometer will cause a serious hazard since uncontained mercury will volatilize very rapidly.
- e. Volatile materials may be present in sufficient concentration to form explosive mixtures within the oven itself. If they are present, vent the oven to an exhaust system to mitigate this hazard.

7.10.2 Hot Plates / Heater-Stirrer Plates

- a. Hot plates / heater-stirrer plates with temperature control devices must be spark-free.
- b. If water or other liquid has been spilled onto the element, have the equipment serviced before use. Allow plenty of time for the hot plate to cool before handling.

7.10.3 Heating devices (such as heating mantles)

- a. Heating devices require auto-transformers to control the temperature. When used with auto-transformers, make certain that the auto-transformer is properly wired and located away from electrical conductors and combustible materials.
- b. Unattended heating devices must be protected with overload circuitry and with a temperature-sensing device that will turn the power off in the event of overheating.
- c. When cooling water is used in connection with heating (as in the condenser of a solvent still), it is necessary to have an automatic device to turn off the power when water flow is interrupted.
- d. Always support the heating mantle with a ring to allow air circulation around it to prevent overheating of the exterior of the mantle. Never support a heating mantle with combustible materials.

7.10.4 Constant Temperature Rooms

Walk-in warm rooms used for incubation or storage of infectious material may be certified as BSL2 facilities even if they do not contain sinks or coat hooks. Work in such rooms shall otherwise follow all the procedures required for BSL2 laboratories.

7.11 LYOPHILIZERS / FREEZE DRYERS

- a. The exhaust from lyophilizer vacuum pumps should be filtered through HEPA filters, passed through a vacuum line trap or vented into a biosafety cabinet.
- b. Use polypropylene tubes in place of glass ampoules for storing biohazardous material in liquid nitrogen. Glass ampoules can explode, causing eye injuries and exposure to the biohazardous material.
- c. Perform the manipulations of ampoules and vials such as filling of infectious specimens, sealing or closing etc., in a biological safety cabinet.

7.12 MICROSCOPES

- a. Tighten caps on flasks of infectious culture before transporting to the microscope.
- b. Use a secondary container to carry infectious cultures in plates or other containers without tight fitting lids to the microscope.
- c. When using the hemocytometer to count cells, disinfect with an appropriate disinfectant after use.
- d. Disinfect the viewing platform of the microscope after each use.
- e. As with all research involving hazardous chemicals, be familiar with any associated chemical and radiological hazards when performing procedures (e.g. in situ hybridizations) for microscopy work.

7.13 MICROTOMES / CRYOSTATS

- a. A microtome is an instrument used for cutting biological materials into thin sections with a sharp blade. A cryostat is a refrigerated chamber, kept at sub-zero temperatures that contain a microtome. Hazards associated with these equipment are injury from cuts / scrapes from the knife blade and exposure to infectious agents present in the tissue.
- b. Before use, it is essential to be trained and proficient in the proper use of the equipment and in the hazards of the materials used with the equipment.
- c. Handle microtome blades with utmost care as they are extremely sharp.
- d. Do not leave a microtome running unattended and never leave the knife on a microtome when not in use. After use, always return the knife to its case.
- e. Wear appropriate PPE such as a lab coat, mask, gloves, safety glasses or goggles to protect from exposure to the materials being used. Surgical grade Kevlar gloves that provide dexterity and cut protection and stainless steel mesh gloves can provide additional protection from cuts and scrapes.
- f. Always use appropriate engineering controls (e.g. hand heel locking device, forceps, tweezers, dissecting probes, and small brushes) when retrieving samples, changing blades or cleaning equipment.
- g. Freezing, fixing and drying do not inactivate some pathogens, so the pathogens that may be present in the tissue should be considered capable of causing infection. Discard all trimmings and loose tissues as biohazard wastes.
- h. After use, decontaminate equipment using an appropriate disinfectant.

7.14 PIPETTES AND PIPETTING AIDS

- a. Never suction or pipette by mouth. Always use mechanical pipetting aid when pipetting infectious materials or toxic chemicals.
- b. Infectious or toxic materials should never be forcefully expelled from a pipette.
- c. Pipette infectious or toxic materials carefully and avoid dripping liquids onto hard surfaces to minimize aerosol formation. Place a disinfectant dampened towel or other absorbent material on the work surface to catch drips. A plastic backed bench paper is suitable for this purpose. Dispose of as infectious waste after use.
- d. Do not mix infectious or toxic fluids by bubbling air from a pipette through the fluid to minimize formation of aerosols.
- e. Dispense liquids from a pipette down the wall of the container instead of dropping from a height. Dispense as close as possible to the contents in the container.

- f. Filtered pipettes and pipette tips that are plugged with cotton or filter should always be used for transferring infectious or toxic materials. Pipetting aids should also have filters to prevent contamination of the device during over-aspiration of liquids.
- g. Place discard pans for used pipettes within the biological safety cabinet. The practice of discarding pipettes outside the biosafety cabinet is not recommended as it provides opportunity for dripping of contents from the pipette and the creation of aerosols.
- h. Place non-disposable contaminated pipettes horizontally into a pan or tray with enough suitable disinfectant to allow complete immersion of the pipettes. After suitable contact time, the pipettes can be autoclaved for reuse.

7.15 REFRIGERATORS, FREEZERS AND COLD ROOMS

- a. All infectious or toxic material stored in refrigerators, cold rooms or deep freezers should be properly contained in sealed primary and secondary containers and clearly labelled with the scientific name of the contents, date stored and the name of the individual who stored them. Unlabelled and obsolete materials should be autoclaved and discarded.
- b. If a cold room is used for storage of or work with infectious material, follow procedures required for BSL2 laboratories when working in the room.
- c. An inventory must be maintained of the refrigerator, freezer or cold room contents.
- d. Refrigerators, deep-freezers and solid carbon dioxide (dry ice) chests should be defrosted and cleaned periodically. After cleaning, the inner surfaces of the cabinet should be disinfected.
- e. Do not store flammable solutions in a refrigerator, freezer or cold room unless it is explosion-proof. Notices to this effect should be placed on refrigerator doors.
- f. Freezers operate at extremely low temperatures of up to -150°C and the interior or contents can cause cold burns if touched with bare skin. Wear appropriate cryogloves when handling materials inside the freezer.

7.16 ULTRAVIOLET (UV) - EMITTING EQUIPMENT

Common UV-emitting equipment encountered in the laboratory includes UV transilluminators and germicidal UV lamps found inside most BSCs.

UV radiation from the equipment can be intense is very damaging to the eyes and skin causing burns to the cornea or skin burns / cancers. Always wear laboratory coats and polycarbonate safety glasses and face shields to protect skin and eyes.

No personnel should work a room where UV lights are on without wearing appropriate PPE.

7.16.1 UV Transilluminators

- a. UV Transilluminators are common equipment in molecular biology laboratories primarily used for the visualization of nucleic acids on agarose or polyacrylamide gels.
- b. Most transilluminator units are supplied with a moveable UV blocking shield and this must always be used in accordance with the manufacturer's instructions.
- c. Transilluminators supplied with digital imaging systems are often interlocked via a micro switch, which turns the unit off when the access door is opened. Do not tamper or by-pass the safety interlock mechanism.

- d. If your transilluminator units do not have any of the safety mechanisms mentioned above, explore the possibility of having them retrofitted by the supplier of the equipment or local specialist workshop staff.
- e. Where possible, use a safe alternative such as dark readers that can be used for visualization of nucleic acids but do not emit hazardous UV radiation.

7.16.2 Germicidal UV lamps in BSCs

- a. Many BSCs are equipped with germicidal ultraviolet (UV) lamps. There is a common misconception that UV sterilization of the BSCs can achieve decontamination. The visible blue-violet glow of the UV lamp does not indicate there is germicidal effect.
- b. The germicidal effect of the UV lamp is limited by a number of factors:
 - *Penetration* – In a dynamic air stream (e.g. biological safety cabinet), UV light is not penetrating. Microorganisms beneath dust particles or beneath the work surface are not affected by the UV irradiation.
 - *Relative Humidity* – Germicidal effect drops drastically above 70% relative humidity, the germicidal effects drops off precipitously.
 - *Temperature* – Temperatures below an optimum temperature of 25°C result in reduced output of the germicidal wavelength.
 - *Cleanliness* – The UV lamp needs to be cleaned periodically to remove dust as dust and dirt can block the germicidal effectiveness of the ultraviolet light.
 - *Age* – The intensity of germicidal wavelength light (UV-C) emitted from UV lamps decreases with age and bulb ratings (hours of use). UV lamps should be checked periodically (approximately every six months) to ensure the appropriate intensity of UV light is being emitted for germicidal activity.
- c. The Centers for Disease Control (CDC) and the National Institute of Health (NIH) states that UV lamps are neither recommended nor required in biological safety cabinets.
- d. Because UV light overexposure may result in adverse health effects, and the beneficial use of UV to the research may be diminished by the factors listed above, the use of UV in BSCs should be carefully considered and avoided.
- e. If installed, UV lamps must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the ultraviolet light. The lamps should be checked periodically with a meter to ensure that the appropriate intensity of UV light is being emitted. A sign must be placed on the entrance door when a UV lamp is operating. UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer.

7.17 MISCELLANEOUS EQUIPMENT (WATERBATHS & SHAKERS)

7.17.1 Waterbaths

Waterbaths used to inactivate, incubate, or test infectious substances should contain a disinfectant as pathogenic or nonpathogenic agents may contaminate water baths. For cold water baths, 70% propylene glycol is recommended. A beaker can be used to contain your samples and any potential leaks from contaminating the entire water bath. To prevent electrical shocks, unplug the unit before filling or emptying and have the continuity-to-ground checked on a regular basis.

7.17.2 Shakers

Shakers should be examined carefully for potential breakage of flasks or other containers being shaken. Screw-capped durable plastic or heavy walled glass flasks should be used. These should be securely fastened to the shaker platform. An additional precaution would be to enclose the flask in a plastic bag with or without an absorbent material. It is also good practice to monitor the shaker when it reaches its set speed to check if the flasks are adequately secured before leaving.

7.18 RECORDS

- a. Records of equipment certification / maintenance schedules should be kept on file.
- b. Training records on the use and care of equipment.

CHAPTER 8 DECONTAMINATION AND DISPOSAL

Materials containing infectious agents must be decontaminated prior to reuse or disposal. The aim of decontamination is to reduce or eliminate the potential of infectious agents to cause disease. A basic knowledge of disinfection is crucial for biosafety in the laboratory where specific decontamination requirements depend on the type of experimental work and the nature of the infectious agent(s) being handled.

Decontamination is a process that removes and/or kills microorganisms. It can be achieved by sterilization or disinfection. These terms are used synonymously but are distinct from one another:

Sterilization is the process that kills and/or removes all classes of microorganisms and spores.

Disinfection is a physical / chemical means of killing microorganisms, but not necessarily spores.

A **disinfectant** is a chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores and is usually applied to inanimate surfaces or objects.

An **antiseptic** is a substance that inhibits growth and development of microorganisms without necessarily killing them and is usually applied to body surfaces.

8.1 METHODS OF DECONTAMINATION

8.1.1 Heat Sterilization

The application of heat, either moist or dry, is recommended as the most effective method of sterilization. Disinfection as a physical or chemical means of killing microorganisms, but not necessarily spores. This is to distinguish it from sterilization which is defined as a process that kills and/or removes all classes of microorganisms and spores. Although many items can be easily autoclaved, there are situations where autoclaving is not an option, for example when dealing with biological spills, surface decontamination and materials that cannot withstand the steam autoclaving conditions.

- a. **Dry heat** at 160°C to 170°C for periods of two to four hours is suitable for destruction of viable agents on an impermeable non-organic material such as glass, but is not reliable in even shallow layers of organic or inorganic material that can act as insulation.
- b. **Incineration** is another use of heat for decontamination. Incineration will burn any organism to ash. It serves as an efficient means of disposal for human and animal pathological wastes.
- c. **Steam heat** via the use of an autoclave is a widely used method for heat sterilization. **Autoclaving** is the most convenient method of rapidly achieving sterility under ordinary circumstances as moist heat causes the denaturation of proteins at lower temperatures and shorter times than dry heat.

Autoclaves can sterilize all items that are heat stable. Proper autoclave treatment will inactivate all fungi, bacteria, viruses and also bacterial spores, which can be quite resistant. Solid surfaces are

effectively sterilized when heated to 121°C, at 15 psi for at least 15 minutes. Liquids and instruments packed in layers of cloth require a much longer time to reach a sterilizing temperature.

Laboratory personnel should be aware of the safe and proper operation of autoclaves. See Section 8.5 on the 'Safe Operation of Autoclaves'.

8.1.2 Liquid Chemical Disinfectants

Contact times for disinfectants are specific to each biological agent, buffer or culture media constituents and the class of disinfectants. The generic discussion on disinfections in this section aims to provide a framework to develop both standardized and more specific procedures to address biohazards in the biomedical laboratory.

- a. Liquid chemical disinfectants can be used for surface decontamination and, at sufficient concentration, as decontaminants of liquid wastes for final disposal in sanitary sewer systems. However, proper consideration must be given to such factors as temperature, contact time, pH, the presence and state of dispersion, penetrability and reactivity of organic material at the site of application in order for decontamination to be effective.
- b. Most chemical disinfectants are not sterilizers and should not be relied upon to destroy all organisms on a surface or piece of equipment. Simple wiping of the surface to be decontaminated with a liquid disinfectant does not kill all the organisms present.
- c. Liquid disinfectants can be categorized as halogens, acids and alkalis, heavy metal salts, quaternary ammonium compounds, phenols, aldehydes, ketones, alcohols, and amines and are commonly available in a variety of trade names.

8.1.3 Disinfectant Guidelines

Based on the recommendations from the Healthcare Infection Control Practices Advisory Committee (HICPAC) for the Centers for Disease Control and Prevention (CDC), an ideal disinfectant should have the following properties:

- a. Broad Spectrum - where it is active against a large repertoire of microbes;
- b. Fast Acting - where it should rapidly kill the microbes;
- c. Unaffected by environmental factors – where it retains its efficacy in buffers;
- d. Non-toxic - where it should not be harmful to users;
- e. Surface compatibility – where it should not corrode instruments and metallic surfaces and not cause the deterioration of cloth, rubber, plastics and other materials;
- f. Residual effect on treated surfaces – where it should leave an antimicrobial film on the treated surface;
- g. Odourless – where the odour is not unpleasant;
- h. Economical – where repeated use is not prohibitively expensive;
- i. Stable – where it does not degrade in normal storage and
- j. Environmentally friendly – so as to facilitate disposal.

Unfortunately, commercial disinfectants do not satisfy all the above criteria, so the following section will provide information on the modes of action of a few commonly used disinfectants.

8.1.4 Classes of Liquid Disinfectants

Disclaimer: Reference herein to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favouring by OSHE.

- a. **Alcohols**, such as ethanol or isopropyl alcohol in concentrations of 70%, are effective against vegetative bacteria and enveloped viruses, less active against non-enveloped viruses and not effective against bacterial spores. They are not significantly harmful to personnel using them but can dry the skin if used regularly on the skin. Alcohol is highly flammable and air-alcohol mixtures can cause a flash fire. It can also be a respiratory and mucosal membrane irritant if sprayed in large amounts. Use only in small amounts and keep open flames away. Do not operate electrical switches nearby. The volatility of alcohol-based disinfectants prevents the recommended contact time for hard surfaces unless reapplied. They can be useful if instruments are soaked in them for 20 minutes or more, given their limited range of effectiveness. The disadvantages are the slow germicidal action and its poor penetrative action where the organic load is high and thus not a good choice for biological spills containing proteins, cell debris, supplements, etc.
- b. **Oxidizers include halogens** (e.g. chlorine and iodine) as well as **peroxymonosulfates** that is the active component found in Virkon.
 - **Chlorine-containing solutions** commonly available as household bleach are active against bacteria, fungi and viruses. At higher concentrations and extended contact times, chlorine can inactivate bacteria spores as well. However, they are corrosive to metals and tissues. Chlorine-based disinfectants contain either sodium hypochlorite (NaOCl) or sodium dichloroisocyanurate (NaDCC) as the active compound. These are popular amongst laboratory users due to the fast killing action via oxidation as well as being effective against a broad spectrum of microbes. It is commonly available as household bleach with 5% sodium hypochlorite (NaOCl), while alternatives in the form of solid powders or tablets are also available to maintain activity before being dissolved in the appropriate concentration. This class of disinfectant is corrosive to metal surfaces and such surfaces should be rinsed or wiped with clean water after disinfection to mitigate its corrosive effects. As a precaution, chlorine-based disinfectants must not be mixed with acids, products containing ammonia or autoclaved, as toxic chlorine or ammonia gas can be released. Also, carcinogenic products are produced when mixed with formaldehyde.
 - **Iodophors**, which are iodine containing formulations, are active against vegetative forms of bacteria, fungi and viruses. There are non-toxic to humans and are generally used as antiseptics and in surgical soaps. Iodine and iodophors, such as iodine, are not high-level disinfectants because of their variable efficacy against bacterial endospores and some fungi. Also, *Pseudomonas* bacteria have been known to multiply in iodophors.
 - **Peroxymonosulfates**, most commonly available as the active component in Virkon, has a wide spectrum of activity against viruses, some fungi, and bacteria and are effective in the presence of organic matter. It is sold as tablets or powder that dissolve readily in water and is intended to be mixed with water to form a 1% to 3% solution (by weight, i.e. 10g to 30g per litre). The pink colour is useful in gauging the concentration of the active ingredients as the Virkon changes to yellow as it ages, making it obvious when it needs to be replaced. The solution is generally stable for five to seven days. Virkon has a faint raspberry odour,

but the scent is still considered unpleasant by some. Virkon disinfectant does not typically cause skin irritation/corrosion, but can cause eye damage and should not be used as a hand-washing liquid. The powdered form may irritate eyes and the respiratory tract.

- **Hydrogen peroxide** and **peracids** are strong oxidizers like chlorine and are as effective as chlorine-based disinfectants. Peracids have very strong, irritating vapors and respirators and strong ventilation are used when applying them. However, hydrogen peroxide in liquid form requires a long contact time to be effective and can be corrosive and unstable. It is therefore not used for hard surface disinfection unless it is vaporized (see discussion below).
- c. **Phenolic-based compounds** are effective decontaminants against some viruses, fungi, and vegetative bacteria, including rickettsiae. They are not effective in ordinary use against bacterial spores. Phenolic compounds (e.g. Triclosan, some formulations of Lysol) are active against vegetative bacteria but not active against spores and have variable effect on non-lipid viruses. These disinfectants maintain their activity in the presence of organic material. However, they can be inactivated in hard water, may be absorbed by rubber and can also penetrate skin.
- d. **Quaternary Ammonium Compounds** are cationic detergents that are strongly surface-active. They are only effective against vegetative bacteria and lipid-containing viruses. They are easily inactivated by the presence of excess organic material. Quaternary ammonium compounds (e.g. benzalkonium chloride) are often used as part of a mixture in commercial disinfectants but pose environmental concerns due to their poor biodegradability. Their activity can also be greatly reduced by organic compounds, water hardness and anionic detergents. There are some recent formulations that are more resistant to organic matter.

8.1.5 Vapours and Gases

Chemical decontaminants that are gaseous at room temperature are useful as space-penetrating decontaminants. When employed in a closed system and under controlled conditions of temperature and humidity, excellent decontamination can result. Common ones are formaldehyde, ethylene oxide, vaporized hydrogen peroxide (H_2O_2) and chlorine dioxide (ClO_2). When performing any decontamination (whether in a BSC or room) the user must evaluate what is necessary for an effective decontamination. What must be recognized is that all decontamination methods can work if the agent reaches ALL surfaces for a prescribed amount of time and at the correct concentration. Thus the following have to be considered:

- Good and complete distribution;
- Thorough and total penetration;
- Sufficient contact time at the specified concentration.

Any decontamination method requires a complete and thorough distribution of the sterilant to get an effective decontamination. For a BSC, the agent used must get to all corners of the work area, it must get below the work surface, it must get to the plenums, it must get through the HEPAs, and it must get to the clean side of the exhaust HEPA.

When a large piece of equipment, like a biological safety cabinet, or a room needs to be decontaminated thoroughly, gases or vapors are usually used and the equipment or room is sealed to prevent any leakage of the gas/vapour. Such chemicals should only be used by licensed or certified personnel or vendors and verification of the effectiveness must be monitored with the appropriate biological indicators. Avoid inhalation of vapours of the decontaminants.

Vapour and gas decontaminants are primarily useful in decontaminating biological safety cabinets, bulky or stationary equipment that resists penetration by liquid surface decontaminants; instruments and optics that may be damaged by other decontamination methods; rooms, buildings and associated air-handling systems and air filters. Gaseous decontamination is not normally used in labs except for BSL3 and BSL4 labs under particular circumstances (e.g. after a spill or accidental release of infectious materials, for removal of large equipment items from containment, before maintenance work on contaminated systems, before retesting of HVAC control systems).

8.1.6 Classes of Vapour / Gaseous Disinfectants

- a. **Formaldehyde (HCHO)** is a pungent, toxic gas that can irritate eyes and mucous membranes. Depolymerized paraformaldehyde as a gaseous sterilizing agent is prepared by heating of solid paraformaldehyde. This is typically used to fumigate biological safety cabinets and enclosed rooms where it effectively inactivates all microorganisms and spores at room temperature as it achieves good penetration. However, it is considered a carcinogen, so personnel exposure must be limited and considerable care is required when handling, storing and using formaldehyde. It is not practical to use it on a daily basis. It must be neutralized at the end of the treatment and its residue removed from hard surfaces as it can continue to give off gas. Formaldehyde in solution form as formalin is used as a fixative and liquid sterilizing agent, provided that the immersion time is sufficiently long.
- b. **Vapourized Hydrogen Peroxide (H₂O₂) or Vapour Phase Hydrogen Peroxide (VPHP):** The vapour is generated by specially designed equipment that boils/vapourizes 31%-35% liquid hydrogen peroxide. These generators initially dehumidify the ambient air, then produce VHP by passing aqueous hydrogen peroxide over a vapourizer, and circulate the vapour at a programmed concentration in the air, typically from 140 ppm to 1400 ppm depending on the infectious agent to be cleared. By comparison, a concentration of 75 ppm is considered to be "Immediately Dangerous to Life or Health" in humans. After the VHP has circulated in the enclosed space for a pre-defined period of time, it is circulated back through the generator, where it is broken down into water and oxygen by a catalytic converter, until concentrations of VHP fall to safe levels (typically <1 ppm). Alternatively, the VHP is vented to the outside air, in cases where recapturing of the VHP is not needed. VHP leaves no residues as compared to formaldehyde, and it is considered to be non-carcinogenic. VHP has been proposed as a safer alternative compared to gaseous decontamination with formaldehyde due to its "residue free" nature (the only residues are oxygen and water). Vapourized hydrogen peroxide is a strong oxidant and a broad spectrum, non-destructive sterilant for the decontamination of rooms or laboratory equipment.
- c. **Chlorine dioxide (ClO₂) gas** is a strong oxidizing agent and effective disinfectant used to sterilize medical and laboratory equipment, surfaces and rooms. It is effective against spore-forming bacteria. As ClO₂ is a true gas, it has much better penetration abilities for the decontamination of isolators, piping systems, pass-throughs, rooms, HVAC duct work, floor drains or cabinets. Classified as a "sterilizer", chlorine dioxide is a yellow-green gas with an odor similar to chlorine but otherwise it is very different. It has been recognized as a disinfectant since the early 1900s and has been approved by the US EPA for many applications. It has been demonstrated effective as a broad spectrum, anti-inflammatory, bactericidal, fungicidal, and virucidal agent, as well as a deodorizer. Pure chlorine dioxide is an unstable gas and therefore is generated as needed. Although chlorine dioxide has "chlorine" in its name, its chemistry is radically different from that of chlorine, being less reactive with ammonia or most organic

compounds. Chlorine dioxide oxygenates products rather than chlorinating them and hence does not produce environmentally undesirable organic compounds containing chlorine. In addition, chlorine dioxide gas does not leave a residue and does not require a post treatment wipe down. Chlorine dioxide gas has a discernible odour at safe levels (0.1 ppm odour threshold), meaning one is able to smell the gas before it reaches unsafe concentrations (0.3 ppm) at 15 minute short term exposure limit (STEL). Typical spaces decontaminated by chlorine dioxide are animal housing Rooms, laboratories, surgical suites, decontamination chambers, heating air conditioning ducts, HEPA filters, and isolator.

When a high level of disinfection is needed, for instance instruments which enter sterile body sites, and the instruments cannot be autoclaved for sterilization, they can be treated with the following chemicals:

- **Glutaraldehyde ($\text{OHC}(\text{CH}_2)_3\text{CHO}$)** is a broad spectrum disinfectant and may require some form of alkaline activation before use. It is toxic and an irritant to skin and mucous membranes and is thus not recommended to use as a spray or as a solution on environmental surfaces.
- **Ethylene oxide** use is very limited and is generally used in surgical and clinical areas and for sterilizing disposable medical devices. Ethylene oxide however is highly flammable and is mutagenic. It requires a longer time to sterilize than any heat treatment and the process also requires time for aeration post sterilization to remove toxic residues.

8.1.7 Radiation

Sterilization can be achieved using radiation such as gamma rays, X-rays or ultra-violet (UV) radiation.

- a. **Gamma rays and X-rays** are sources of ionizing radiation active against bacteria, viruses and spores and are commonly used for sterilization of prepackaged disposable medical devices, such as syringes, needles. Gamma rays are very penetrating and require bulky shielding operator safety. X-rays are less penetrating but require less shielding. Ionizing radiation is not a practical tool for the laboratory.
- b. **Ultraviolet light irradiation** is useful only for sterilization of surfaces and some transparent objects. However, because this process is not fully controllable, and UV light can cause harm to unprotected human skin or plastics, the use of UV for surface decontamination is discouraged in biological laboratories. The Centers for Disease Control and the American Biological Safety Association do not endorse the use of UV light as a surface disinfectant or its use in biological safety cabinets.

8.2 SELECTING CHEMICAL DISINFECTANTS

8.2.1 General Considerations for Selecting Chemical Disinfectants

- a. Microorganisms exhibit a wide range of resistance to inactivating agents. Most vegetative bacteria, fungi and lipid-containing viruses are relatively susceptible to chemical decontamination whereas non-lipid containing viruses and bacteria with a waxy coating e.g. tubercle bacillus have mid-range resistance. Spores are most resistant to inactivation (**Appendix B.1**).
- b. No single chemical disinfectant or method is effective for decontamination in all situations. The choice of chemical disinfectants should be made after consideration of the following factors:
 - Target organism(s)
 - Highest concentration of organisms
 - Amount of extraneous organic material present
 - The material & area to be decontaminated
 - Application method, contact time possible
 - Potential toxicity of disinfectant
 - Activity of disinfectant
 - Stability, storage conditions

Please refer to **Appendix B.2** for more information.

These factors are different for a routine cleanup / decontamination procedure after tissue culture work in a BSC as compared to a 5 liter spill from a pilot fermenter. Consequently, different disinfectants or concentrations may be used in different situations where the presence of high organic load would require more concentrated or harsher disinfectants than those used for surface cleaning.

8.2.2 Evaluating Commercial Disinfectants

Most manufacturers will provide a list of biological agents that their product can inactivate. Use disinfectants that are registered with a regulatory organization as these products are effective against human blood borne pathogens. Most product sheets will also include recommendations on the concentration and contact time required for different classes of pathogens. There are also peer-reviewed publications on the efficacies of disinfectants and these can be used as a resource for the choice of disinfectants, their working concentrations and contact time.

The various regulatory authorities in Europe (e.g. European Standard or Norm, EN; British Standards, BS; Germany, DGHM; France, AFNOR) or North America (e.g. Food and Drug Administration, FDA; Environmental Protection Authority, EPA; Association of Official Analytical Chemists, AOAC) have all developed standardized efficacy tests for disinfectants which are quite similar.

- a. **Suspension tests** focus on viable counts performed following neutralization of residual disinfectant either by dilution or by adding specific neutralizers (Appendix 8.3). Negative controls are required to account for variations in serial dilutions and the bactericidal effect can be expressed as $B_E = \log N_C - \log N_D$ where N_C and N_D represent the final number of CFU/ml remaining in the control and disinfectant series, respectively. The limitation of this method lie with disinfectants that promote clumping where the enveloped bacteria may be protected although this may be overcome by adding non-ionic surfactants to the diluent.
- b. The **in-use method** is primarily used to determine the efficacy of disinfectants in discard jars where the final CFU/ml is determined and more than 10 colonies would suggest poor-killing effects. Similarly, the **simulated use method** involves contaminating surfaces with bacteria

suspensions, allowing the surface to dry and then exposing to the disinfectant. The microbes are then swabbed off the surface and resuspended in suitable neutralizing medium and assessed for viability as for suspension tests (see **Appendix B.3**).

- c. **Fungicidal tests** recommend using spore suspensions (in saline containing the wetting agent Teen 80) obtained from 7-day-old cultures of at least 10^6 CFU/ml. Viable counts on suitable media (e.g. malt extract agar) with incubation at 20°C for 48 hours or longer. EN 1275 regulations for fungicidal activity require a minimum reduction of in viability by a factor of 10^4 within 1 hour using *Candida albicans* and *Aspergillus niger*.
- d. **Antiviral tests** are not so easily accomplished due to the requirement for suitable host cells. In general, the virus is grown in an appropriate cell line that is mixed with water containing an organic load and the disinfectant. After an appropriate contact time, residual viral infectivity is determined using plaque assay or other systems (e.g. animal host, molecular assay for viral components). Such procedures are costly and time-consuming and must include controls to exclude factors such as disinfectant killing of the cell system or test animal. A reduction of infectivity by a factor of 10^4 is regarded as evidence of acceptable virucidal activity (EN 14476).
- e. Users are encouraged to check the product sheet of commercial disinfectants for compliance with recognized standards for validation of the efficacy. If there is any doubt, users can consider sending a testing request to accredited laboratories which use standards such as ISO / IEC 17025, etc.

8.3 GUIDELINES FOR USE OF COMMONLY USED DECONTAMINANTS

Sterilization and decontamination are dynamic processes which are time-dependent. The *contact time* is crucial for effective killing of the target organisms. The time needed depends on the agent or method, cleanliness and accessibility of surfaces, and to the resistance of the agent.

Decontaminants / disinfectants should be used in accordance with manufacturer's directions on the label and in the product sheet to ensure effective decontamination. In doubt, please contact your Safety and Health Officer for more information.

A decontaminant selected on the basis of its effectiveness against organisms on any range of the resistance scale will be effective against organisms lower on the scale i.e. disinfectants that effectively control spore forms can be assumed to inactivate any other organism.

High titers of microorganisms or the presence of large amounts of organic materials such as agar, proteinaceous nutrients, and cellular materials can effectively retard or chemically bind the active moieties of chemical disinfectants. Such interference with the desired action of disinfectants require higher concentrations and longer contact times and may not be directly proportional, i.e. doubling the organic load may require more than twice the contact time and/or concentration of disinfectant used.

A decontaminant can be rendered ineffective as a result of failure of the decontaminant to contact the microorganisms. Microorganisms in biofilms, under spots of grease, rust, dirt or dry areas of tiny bubbles on the surface of the item may not be affected by the decontaminant.

The more active the disinfectant, the more likely it will possess undesirable characteristics such as corrosiveness. Particular care should be observed when handling concentrated stock solutions of

disinfectants. Personnel should be aware of safety precautions to follow and appropriate personal protective equipment to use when handling them.

8.3.1 Hypochlorites

Hypochlorites (e.g. bleach and Presept) are broad-spectrum disinfectants used in many laboratories here. It is active against bacteria, fungi and viruses. At higher concentrations and extended contact times, bleach can inactivate bacteria spores as well.

- a. Guidelines for use:
Liquid wastes can be decontaminated with 1:10 final dilution of household bleach (i.e. one part bleach to 9 parts liquid) for 30 minutes. A lower dilution (higher concentration) should be considered if there is much organic matter in the liquid (i.e. tissue culture waste). After decontamination, liquid waste can be disposed of in the public sewer with copious amount of water provided no other hazardous materials are present (e.g. chemicals and/or radioactive materials).
- b. Effective working concentrations of hypochlorite for disinfection are:
 - “Dirty” conditions (e.g. presence of large amounts of organic matter) - Sodium hypochlorite solution containing 0.5% available chlorine (equivalent to 5 grams per litre or 5000 parts per million)
 - “Clean” conditions (e.g. for disinfecting surfaces, rinsing protective clothing) - Sodium hypochlorite solution containing 0.1% available chlorine (equivalent to 1 gram per litre or 1000 parts per million)

Domestic household bleach is typically made of 5.25% (52,500 ppm). Sodium hypochlorite but can range from 3-6%. Industrial bleach solutions have a higher concentration (10-15% sodium hypochlorite). They have to be diluted accordingly to obtain the working concentration. Please consult the product sheet of commercial solid chlorine-releasing disinfectants (e.g. Presept) for the equivalent availability of chlorine to add per volume of liquid.

- c. The efficacy of hypochlorites to act as a disinfectant is considerably reduced:
 - by presence of organic material (e.g. serum and protein in blood);
 - with storage of ready-made solutions;
 - by exposures to high temperature, oxygen and sunlight.
- d. Hypochlorite concentrations drop over time due to relative instability of the active chlorine component. As a general guide, solutions with high levels of organic matter (i.e. such as tissue culture waste) should be changed at least daily, while those with less frequent use may last for as long as a week.
- e. Hypochlorite solutions can also be made from:
 - *Bleach powder* - Chlorine compounds available in powder form (e.g. calcium hypochlorite); or
 - *Chlorine-releasing tablets* - Sodium dichloroisocyanurate, or commercial preparations e.g. “Presept” or “Haz-Tab” tablets.

Solutions can be made freshly for use when required. Follow the manufacturer's instructions for the preparation and use of working solutions.

- f. Many by-products of chlorine can be harmful to humans. Avoid indiscriminate use of chlorine-based disinfectants and follow safety precautions when using bleach:
- Chlorine gas is highly toxic. Store and use bleach in a well-ventilated area.
 - Household bleach containing 5% sodium hypochlorite is considered to be an irritant. More concentrated bleaches containing 10-15% sodium hypochlorite are considered corrosive. Avoid direct contact with skin and eyes. Contact with skin can produce caustic irritation or burns. Splash goggles/face shield and protective gloves are recommended PPE when handling, diluting, etc.
 - Hypochlorite and other chlorine-releasing disinfectants may cause corrosion of metals and this must be taken into account when decontaminating equipment. It is good practice to wipe down with water to remove residual hypochlorite on metal surfaces.
 - Products containing chlorine dioxide or other forms of chlorine can successfully and safely be used in BSCs constructed with a high quality (grade 16 or higher) of stainless steel if the chlorine residue is rinsed off with sterile water or 70% alcohol after the effective contact time.
 - Do not mix hypochlorites with other chemicals. For example, bleach mixed with acids or ammonium-containing materials rapidly generates the toxic chlorine and chloramine gas respectively. Check the incompatibility chart of bleach.
 - Do not autoclave solutions containing bleach as toxic and corrosive chlorine gas can be liberated.

8.3.2 Peroxymonosulfates

Potassium peroxydisulfate (e.g. Virkon) is a multi-purpose disinfectant typically used for cleaning up hazardous spills, disinfecting surfaces and soaking equipment. The solution is used in many areas, including hospitals, laboratories, nursing homes, etc. where disinfection is required. It is a broad-spectrum disinfectant but is less effective against spores and fungi. Please refer to the [Virkon label](#) for the microbes that Virkon has tested as being effective against.

- a. Guidelines for use:
Biological spills can be overlaid with Virkon powder for a contact time of 10 minutes before scraping the mixture into a safe receptacle as disposed through licensed Toxic Industrial Waste contractors. The surface should be followed up by washing with 1% Virkon. Routine disinfection of hard surfaces can be accomplished using 1% Virkon followed by rinsing with water after 10 minutes. Avoid using Virkon on acid-sensitive surfaces such as copper, brass or aluminum.
- b. The effective concentration and contact times for Virkon varies between 1-3% and up to 1 hour. The University of Edinburgh recommends the following conditions:

Table 8.1 Recommendation on effective concentration and contact times for Virkon

Item	Virkon Concentration	Contact Time
Plasticware	1% solution	at least 2 hours fully immersed
Liquids e.g. samples, culture supernatants, etc.	final concentration of 2% when liquid is added	at least 2 hours
Surfaces including benches and floors	1% solution	wipe over

Minor surface contamination	1% solution	10 minutes
Larger spillage	granules	10 minutes

- c. Virkon should be freshly prepared and the pink coloration is an indicator of its activity that typically lasts for a week.
- d. As with all disinfectants, its efficacy is reduced:
 - In the presence of organic material (e.g. serum and proteins in blood)
 - With prolonged storage of the working solution
 - By exposure to high temperature, oxygen and sunlight.
- e. Please be careful with Virkon powder as it is corrosive and irritates the respiratory tract and mucosal membranes. Tablet forms are recommended. However, if powders are preferred or used to soak up a spill, small packs (<10 pounds) can be used to minimize the volume of powder handled.
- f. Virkon is incompatible with strong alkalis and releases toxic halogen gases such as chlorine, bromine and iodine in the presence of halogen salts (e.g. KCl, KBr, KI, NaCl).

8.4 BIOLOGICAL WASTE DECONTAMINATION AND DISPOSAL

Depending on the category, wastes in the University containing biological agents is either sterilized and disposed off as regular wastes, or collected by licensed biowaste collectors.

Wastes from laboratories may include infectious wastes, pathological wastes, contaminated sharps, routine clinical wastes, cytotoxic wastes, radioactive wastes, pharmaceutical wastes, chemical wastes and general wastes. Wastewater generated from biological /food analysis labs can be discharged directly into the sewer after dilution.

8.4.1 Biowaste Classification

- a. Biowaste shall be classified based on their treatment or disposal methods (refer to **Appendix B.4**).
- b. The Faculty Safety & Health Officer or the Office of Safety, Health and Environment (OSHE) shall assist the Principal Investigator in determining waste and hazard classification, if required.
- c. The waste container/bottle shall have caution labels indicating the hazard classification and class of waste.
- d. Except for sharps and liquid wastes for disposal into sewer, all biohazard wastes are to be placed in yellow plastic bag with biohazard sign printed on it. Sharps shall be placed in appropriate sharps containers.
- e. If the biological waste is contaminated with chemical agents, the waste shall be treated as chemical waste.

8.4.2 Biowaste Disposal

- a. All wastes containing biohazardous material should be handled with gloves. Contaminated lab coats, dirty gloves and other contaminated materials must be autoclaved. To assure adequacy

of sterilization, autoclaves must be tested routinely with spore strips and other methods as recommended by the manufacturer. Refer to OSHE's guidelines on Safe Operation of Autoclaves in Section 8.5.

- b. Free flowing liquid waste e.g. cultures of microorganisms, tissue culture wastes, shall not be disposed off with solid waste nor discarded down the drainage system. The waste shall be contained in leak proof, rigid durable containers labeled with the biohazard symbol and the word "biohazard". Liquid wastes shall be decontaminated by autoclaving or with the use of an appropriate chemical disinfectant in accordance with the manufacturer's recommendations. The treated waste can then be disposed off in the sewer system.
- c. Sharps shall be placed in appropriate sharps containers that are labeled "biohazard".

8.4.3 Sharp-Waste Disposal

- a. Sharps pose a physical-injury hazard as well as an infection hazard and a high degree of precaution should be taken. These include blood-drawing equipment, needles, syringes, slides, Pasteur pipettes, capillary tubes, broken glass and scalpel blades.
- b. Biologically contaminated sharps wastes are to be handled separately and differently from the ordinary trash. All contaminated sharps are to be treated as infectious and disposed of only through licensed biohazardous waste collectors.
- c. Contaminated needles should not be broken or clipped unless the clipping device can effectively control the aerosol generation. Needles shall not be recapped or separated from syringes prior to disposal.
- d. Containers for sharps (sharps containers) should be impervious, rigid and puncture proof. Sharps containers shall not be filled beyond the recommended fill line.
- e. Sharps containers shall remain closed at all times except when sharps are being deposited into the container. Sharps containers shall not be placed on the floor in the lab at all times.
- f. Filled sharps containers should be sealed, labeled as 'SHARPS' and the biohazard symbol before disposal by licensed biohazardous waste collectors.

8.4.4 Autoclavable Material

- a. All laboratory specimens or materials consisting of, containing, or contaminated with blood, plasma, serum, urine, faeces or other human or animal tissues or fluids, as well as inoculated media, cultures, and other potentially infectious materials, may be sterilized by autoclaving. Refer to OSHE's guidelines on Safe Operation of Autoclaves in Section 8.5. If autoclaving is not performed, the waste must be sent to NEA-licensed bio-hazardous waste collectors for incineration.
- b. Contaminated solid wastes such as cloth, plastic and paper items e.g. wrappers and paper towels must be put into autoclavable biohazard bags and may be autoclaved.
- c. Sterilization of biological wastes by autoclaving should be monitored using a biological indicator (such as 3M™ ESPE Attest™ Biological Monitoring System, code 1262P) to ensure quality assurance, and must be performed periodically if the autoclaved waste is disposed of as general waste at least once a month.
- d. Autoclaved waste is no longer considered hazardous and can be disposed of as general waste. Solid wastes shall be disposed of ONLY in black or blue trash bags. Autoclaved liquid wastes can be discharged into the sewer with copious amount of water.
- e. Dry hypochlorite or other strong oxidizers should not be autoclaved with organics such as paper and oil. This may lead to explosion.

8.4.5 Wastes for Incineration or Cremation

- a. Animal carcasses, human tissues, organs and sharps wastes are to be disposed of by incineration or cremation through licensed waste contractors.
- b. Waste generated from the use of scheduled agents listed under the Biological Agents and Toxins Act must be sent for incineration by an authorized waste collector / disposal company.

8.4.6 Chemical Decontaminated Wastes

- a. Wastes which cannot be autoclaved should be chemically decontaminated.
- b. Swabs and other disposables soaked in a disinfectant may be double-bagged and treated routinely as general garbage. When using a disinfectant, precautions should be taken in accordance with the MSDS to prevent splashing and other harmful exposure.
- c. Chemically decontaminated wastes should be disposed through licensed toxic industrial waste collectors if the chemicals found in the waste pose a hazard to handlers.

8.4.7 Non-Infectious and Environmentally Benign Wastes

Materials that can be directly discarded into the sewer include uninoculated liquid medium, tissue culture medium, and nutrient fluids, provided these materials do not contain any infectious agent.

8.4.8 Segregation and Storage

- a. All infectious waste containers should be properly sealed and marked with the biohazard label. The Principal Investigator or his designate shall ensure that all wastes are segregated and stored at the designated storage areas. Waste Storage Locations are to be confirmed with the assistance of Faculty Safety & Health Officer. The PI and staff shall ensure good housekeeping for all biological wastes stored in the common area under their jurisdiction.
- b. Prior to entering into or renewing any contract with a waste collector, a Competent Person, or Faculty Safety & Health Officer shall ensure that the waste collector has a valid license for the type of waste to be collected from NUS. The Competent Person shall arrange for licensed toxic and industrial waste (TIW) collectors to collect hazardous waste and TIW when necessary.
- c. A consignment note shall be completed for all hazardous waste and TIW collected. Consignment notes for TIW shall be submitted to NEA's Pollution Control Department (PCD) through e-tracking or mail.
- d. Transport approval from PCD is required for consignment of TIW exceeding the prescribed quantity under Environmental Public Health (TIW) Regulations.

8.4.9 Records

The Competent Person shall maintain copies of all consignment notes. Notes shall be kept in record for at least five years.

8.5 SAFE OPERATION OF AUTOCLAVES

Autoclaves are pressurized equipment used for wet heat sterilization. The autoclaving process commonly use steam heated to 121°C, at 101 kPa above atmospheric pressure for 15 minutes.

Autoclaves are generally used to sterilize instruments, media and glassware, and to decontaminate wastes containing biological agents prior to disposal.

As an autoclave uses saturated steam under high pressure to achieve sterilizing temperatures, proper use is important to ensure operator safety. Appropriate personal protective equipment should be worn when operating autoclaves and when handling potentially infectious materials to be autoclaved.

Preventative maintenance and quality control checks should be done to ensure proper performance of the equipment. A biological indicator kill-test (spore test) should be performed at least once every month in autoclaves processing biohazardous waste. All autoclaves must also be inspected by MOM authorized boiler inspectors yearly or bi-yearly depending on the capacity of the autoclave.

Autoclave tapes serve to identify goods that have been autoclaved. However, the change in colour is not a proof of successful sterilization process, as the tape can sometimes change color at room temperature.

8.5.1 Preparation and Loading of Materials

- a. Use only autoclavable type of materials such as polypropylene, borosilicate (Pyrex) glass or stainless steel. Biological wastes should be autoclaved in biohazard waste bags that are autoclavable.
- b. Ensure materials do not contain materials that are incompatible for use in an autoclave such as solvents, oxidizers, chlorine-releasing compounds and water sensitive chemicals.
- c. Ensure the drain screen is clear of debris to allow proper circulation of steam.
- d. Before using the autoclave, check inside the autoclave for any items left by the previous user that could pose a hazard (e.g. sharps). Please remember to sign the log book to keep track of the total use of the equipment for maintenance purposes, etc.
- e. Add deionized water to the autoclave chamber until the water level reaches just under the tray / basket in the bottom of the autoclave
- f. Liquid containers should not be filled to more than 2/3.
- g. All container caps should be loosened and containers should have vents for release of pressure.
- h. Always put biohazardous waste bags onto secondary container pans or trays to catch spills.
- i. Position biohazard bags on their sides, with the bag neck taped loosely.
- j. Ensure there is adequate space between items to allow for steam circulation.

8.5.2 Cycle Selection for Materials

- a. Use liquid cycle (slow exhaust) when autoclaving liquids, to prevent contents from boiling over.
- b. Select fast exhaust cycle for glassware.
- c. Use fast exhaust and dry cycle for wrapped items.

8.5.3 Time Selection

- a. Take into account the size of the articles to be autoclaved. A 2-liter flask containing 1 liter of liquid takes longer to sterilize than four 500 ml flasks each containing 250 ml of liquid.
- b. Material with a high insulating capacity (animal bedding, high-sided polyethylene containers) increase the time needed for the load to reach sterilizing temperatures.

- c. Bags of biological waste should be autoclaved for a minimum of 30 minutes to ensure decontamination. Materials with high organic load will require longer sterilization times.
- d. Please refer to the user's manual for the time selection. Minimum time for autoclaving cycle should at least be 15 minutes at 121°C.

8.5.4 Removing the Load

- a. The autoclave chamber is a pressure vessel. Never attempt to open the door while the machine is operating. Ensure that the interlock is operational as this is a safety mechanism to prevent opening of the autoclave before the internal temperature drops to less than 60°C.
- b. Check that the chamber pressure is zero (refer to the pressure gauge).
- c. Wear lab coat, eye protection, heat insulating gloves and covered toe shoes.
- d. After the slow exhaust cycle, open the autoclave door.
- e. Stand behind door when opening it.
- f. Slowly open door slightly. Beware of the rush of steam. If you feel any resistance, do not force open the door.
- g. Allow liquids to cool down to ambient conditions before handling them.
- h. For tower style autoclaves, be extra cautious as load removal may require either standing on a step ladder or bending down to reach into the autoclave.

8.5.5 Monitoring & Control

- a. Autoclaves used to decontaminate laboratory waste should be tested periodically to assure effectiveness. The following tests are to be used:
 - Chemical Indicator that fuses when the temperature reaches 121°C; *and/or*
 - Biological Indicator which contains heat-resistant spores (*Bacillus stearothermophilis*) that are killed by exposure to 121°C for approximately 15 minutes.
- b. Both types of tests should be placed well down in the center of the bag or container of waste, at the point closest to the heat.
- c. Chemical indicators should at least be added in one bag per load.
- d. Biological indicator tests must be performed periodically if the autoclaved waste is disposed as general waste, and should be done at least once a month.
- e. If either one of these tests fails, immediately contact the PI, maintenance staff and re-autoclave the waste in another working autoclave.

8.5.6 Safety Certification of Autoclaves

- a. Autoclaves must be subjected to safety (mechanical) inspections by MOM authorized boiler inspectors, by regulation. The frequency of certification, whether yearly or bi-yearly will depend on the capacity of the autoclave.
- b. A current certificate bearing the autoclave number and the Ministry of Manpower (MOM) registration number must be displayed on the autoclave. The certificate must also display the last and the next inspection date.

8.5.7 Other Considerations: Protection from Heat and First Aid

- a. Some older autoclaves have little or no heat shielding around the outside. Attach signs warning of “Hot Surfaces, Keep Away” on or next to the autoclave to remind people of the hazard.
- b. Do not stack or store combustible materials (cardboard, plastic, volatile or flammable liquids) next to an autoclave.
- c. If you receive a minor burn, do the following:
 - Immerse the burn in cool running water immediately (do not wait for ice).
 - Remove clothing from the burn area.
 - Keep the injured area in cool water for at least 5 minutes (longer is better).
 - Report the incident to your supervisor and seek professional medical treatment as needed.

8.5.8 Records

- a. Certificates of inspections by authorized boiler inspectors should be kept by the autoclave owner.
- b. The label of inspection should be displayed at a conspicuous location on the autoclave. Only labels approved by MOM are to be posted. The following must be written clearly on the label:
 - Autoclave serial number
 - Inspection registration number
 - Date of inspection
 - Date of next inspection
 - Name of authorized boiler inspector
- c. Records of spore & chemical (temperature) tests should be kept by the autoclave owner or users.
- d. A record book of the autoclave use should also be maintained.
- e. Training records.

CHAPTER 9 TRANSPORT OF BIOLOGICAL MATERIALS

Shipment of biological materials is regulated locally by government agencies such as the [Ministry of Health \(MOH\)](#) and [Agri-Food and Veterinary Authority \(AVA\)](#) of Singapore. In addition, importation of genetically modified organisms or materials for research purposes is specified by [Genetic Modification Advisory Committee \(GMAC\)](#), in [Singapore Biosafety Guidelines for Research on Genetically Modified Organisms \(GMOs\)](#).

This chapter provides guidelines on the safe transport of regulated as well as non-regulated biological materials between laboratories within the same building, between NUS buildings, into and out of NUS. This is to ensure that the public and the workers in the transportation chain are protected from exposure to any substances that might be in the package.

All biological materials applicable to this chapter are for research purposes only.

9.1 TRANSPORT OF REGULATED BIOLOGICAL MATERIALS

This chapter details how biological materials regulated under the [Biological Agents & Toxins Act 2005](#), [Animals and Birds Act](#) and [Singapore Biosafety Guidelines for Research on Genetically Modified Organisms \(GMOs\)](#) are to be safely packaged and transported within, into or out of NUS to ensure they are in compliance with all national and international regulations. Shipment of biological materials is regulated locally by government agencies such as the MOH and AVA.

International transportation of infectious substances and toxins, including genetically modified micro-organisms and organisms are regulated by the [International Air Transport Association \(IATA\)](#).

9.1.1 Scope

- a. This procedure covers the transport of biological materials within, into or out of NUS, for research purposes only.
- b. All NUS staff and students, and researchers and visitors from other institutions/organizations who have collaborations with NUS, must comply with this procedure.
- c. This procedure covers the transportation of biological agents or toxins regulated by Ministry of Health (MOH) as specified in the [Biological Agents & Toxins Act 2005](#) and subsidiary [Biological Agents and Toxins \(Transportation\) Regulations 2005](#):
 - i. any First Schedule biological agent
 - ii. any Second Schedule biological agent
 - iii. any Third Schedule biological agent transported in quantities aggregating 10 liters or more on any conveyance at any one time
 - iv. any Fourth Schedule biological agent
 - v. any Fifth Schedule toxin
- d. This procedure also covers the transport requirement of veterinary biologics and laboratory animals regulated by Agri-Food and Veterinary Authority (AVA), and transportation for genetically modified organisms and research materials specified in [Singapore Biosafety Guidelines for Research on Genetically Modified Organisms \(GMOs\)](#) (January 2013) by Genetic Modification Advisory Committee (GMAC).

- e. International transport of infectious substances and toxins are regulated by the [International Air Transport Association \(IATA\)](#). The IATA have published the "Dangerous Goods Regulations" which is a manual based on the International Civil Aviation Organization (ICAO) Technical Instructions to provide procedures by which hazardous articles or substances can be safely transported on air.

9.1.2 Definitions

- a. In this procedure, transport refers to the movement of biological materials within, into or out of NUS, including movement of biological materials between research institutes/departments and between laboratories in the same building within NUS, and shipment of biological agents across national boundaries by a commercial conveyance.
- b. "Biological agents" as defined by BATA is:
 - i. any micro-organism (including any bacterium, virus, fungus, rickettsia and parasite);
 - ii. any infectious substance (including any prion); or
 - iii. any component of a micro-organism or an infectious substance (but not including any toxin),

that is capable of causing death, disease or other biological malfunction in a human, and shall be referred to as biological agents which are regulated by Biological Agents & Toxins Act 2005 in this procedure.

- c. "Toxin" as defined by BATA means any poisonous substance that is produced and extracted from any micro-organism, and shall be referred to as toxins which are regulated by BATA in this procedure.
- d. "Veterinary Biologics" as defined by Animals and Birds Act means any viruses, serums, toxins, and analogous products of natural or synthetic origin, including genetically modified organisms, diagnostics, antitoxins, vaccines, live micro-organisms, killed micro-organisms, and the antigenic or immunizing component of micro-organisms intended for use in the diagnosis, treatment, or prevention of diseases of animals and birds, or for purposes of research in animals or birds.
- e. "Inactivated" in relation to a biological agent, means that the biological agent has been rendered non-infectious and unable to replicate itself under any condition.
- f. "Public transportation" as defined by BATA means transportation by bus, taxi, rail or any other conveyance, whether publicly or privately operated, which provides general or special service to the general public on a regular and continuing basis, and includes such other means of transportation as may be prescribed as a type of public transportation for the purposes of this Act.
- g. "Public road" means any public highway or any road over which the public has a right of way or is granted access, and includes every road, street, bridge, passage, footway or square over which the public has a right of way or is granted access.
- h. International Air Transport Association (IATA) Dangerous Goods Regulations (DGR) is regulated by the International Air Transport Association (IATA). These regulations provide packaging and labeling requirements for infectious substances and materials, as well as clinical specimens that have a low probability of containing an infectious substance. These are the

regulations followed by the airlines. These regulations are derived from the Committee of Experts on the Transport of Dangerous Goods, United Nations (UN) Secretariat, and the Technical Instructions for the Transport of Dangerous Goods by air which is provided by the International Civil Aviation Organization (ICAO).

- i. The Exporter / Shipper / Consignor is the party responsible for preparing the materials for transport.
- j. The Shipper / Carrier is the party responsible for transporting the materials from the consignor to the consignee, by rail car, aircraft, motor vehicle, or vessel.
- k. The Importer / Consignee is the person or organization (laboratory, company) shown on a shipping document, package marking, or other media as the location to which a carrier is directed to transport a hazardous material.
- l. Definitions of toxic substances, infectious substances, biological products, cultures, patient specimens, medical or clinical wastes, and genetically modified microorganisms and organisms as specified in IATA are provided in **Appendix C.1**.

9.1.3 Responsibilities

- a. The Director / HOD / PI of the lab performing the transfer / transport has overall responsibility for ensuring that a system is established for the safe transfer or transport of biological materials.
- b. The PI shall hold the responsibilities of the exporter and/or importer for the transport of biological materials that are under his/her supervision.
- c. The Consignor's / Shipper's / Exporter's responsibilities are:
 - i. To classify if a material is a dangerous good or not
 - ii. To select a proper shipping name for the material
 - iii. To ensure the proper packing and packaging material is used
 - iv. To ensure there is proper marking/signage and labeling on the package
 - v. To arrange for the documentation
 - vi. To make arrangements for notifying the importer
 - vii. To update inventory records to reflect transfer of ownership of the materials
 - viii. To be contactable at all times by the carrier or the importer
- a. The Carrier's responsibilities are:
 - i. To ensure compliance with IATA requirements
 - ii. To ensure proper packaging, storage, loading and transport of the container
 - iii. To deliver the package and copy of the shipper's declaration to the consignee
 - iv. To inform the importers in times of delivery failure
- b. The Importer's / Consignee's responsibilities are:
 - i. To inspect packages and report back to shipper
 - ii. To ensure exporters provide proper packaging and labeling
 - iii. To arrange for the most timely and efficient collection on arrival
 - iv. To maintain a copy of the shipper's declaration
 - v. To obtain import and/or other permits
 - vi. To update inventory records to reflect receipt of the materials

- vii. To keep track of the distribution of the human pathogen locally
- viii. To ensure human pathogens are appropriately disposed of after use

9.1.4 Local Transportation of Biological Agents and Toxins Regulated by BATA

Local transportation of biological agents and toxins within Singapore is specified in [Biological Agents and Toxins Act 2005](#) and [Biological Agents and Toxins \(Transportation\) Regulations 2005](#).

9.1.4.1 No person shall transport or procure the transportation of any scheduled biological agents and toxins, including inactivated First and Second Schedule biological agents, within Singapore by mail or public transportation, as specified in Biological Agents and Toxins Act 2005.

9.1.4.2 The Biological Agents and Toxins Act (Transportation) Regulations 2005 has the following additional requirements that apply to the transportation in Singapore of:

- a. any First Schedule biological agent;
- b. any Second Schedule biological agent;
- c. any Third Schedule biological agent in quantities aggregating 10 litres or more carried on any conveyance at any one time; or
- d. any Fifth Schedule toxin

9.1.4.3 Packaging

The packaging of First, Second and Third Schedule biological agents to be transported on public roads in an upright position and shall be triple packaging, which is as follows:

- a. Primary receptacle shall be watertight and leak-proof, wrapped in absorbent material of sufficient quantity to prevent breakage of the primary receptacle and able to absorb all fluids (if any) that may emanate from the biological agent in the event of a breakage of or leakage from the primary receptacle, and be packed in a secondary receptacle.
- b. Secondary receptacle shall be watertight and be packed in a rigid outer container.
- c. Tertiary outer container should be rigid.

The package containing Fifth Schedule toxins to be transported on public roads shall be watertight and leak-proof and be sufficiently strong to withstand any impact which the package would normally be subject to during the transportation, loading or unloading, and shall be transported in an upright position.

9.1.4.4 Labelling

Every primary receptacle shall be affixed with biological hazard signs for First, Second, Third and Fifth Schedule biological agents or toxins, as depicted in **Appendix C.2**.

Every secondary receptacle shall contain an itemized list of the contents in the primary receptacle.

Every rigid outer container shall be labelled with:

- a. the name, address and telephone number of the transferor;
- b. the name, address and telephone number of the transferee;
- c. a 24-hour emergency number that is monitored at all times by a person who —

- (i) has knowledge of the hazards and characteristics of the biological agent or toxins being transported; or
- (ii) has immediate access to a person who possesses such knowledge and information;
- d. the biological hazard signs for biological agents or toxins, respectively; and
- e. a statement that states that the container contains an infectious substance or toxins, respectively.

9.1.4.5 Driver & Vehicle

Every carrier shall ensure that every person employed by him to drive any conveyance used to transport any biological agent or toxin has undergone the relevant training conducted by the Singapore Civil Defence Force, has been issued with a Hazardous Materials Transport Driver Permit by the Singapore Civil Defence Force, and fulfills the following requirements as stipulated in Section 47 of the BATA, such as:

- a. no unreasonable delays in the transportation of any biological agent or toxin;
- b. the driver is trained in the management of accidents involving biohazardous material or other relevant training; and
- c. all necessary steps are taken to ensure the security of the biological agent or toxin in the course of the transportation.

The conveyance used to transport the biological agent or toxin on any public road should be labelled with the biohazard label specified as follows:

- a. Vehicles used to transport biological agents should use the biohazard sign for biological agents
- b. Vehicles used to transport toxins should use the biohazard sign for toxins
- c. Vehicles used to transport a biological agent and a toxin should use both biohazard sign for biological agent and toxin



Biohazard sign for biological agents



Hazard sign for toxins

It is advisable to engage the service of an authorized courier for packaging and transporting your BATA biological agents and toxins safely.

9.1.4.6 International Transport of Biological Agents and Toxins

International transportation of biological agents and toxins are regulated by the International Air Transport Association (IATA). Please refer to the Section 9.1.8 on International Transport of Infectious Substances and Toxins.

9.1.5 Transportation of Veterinary Biologics Regulated by AVA

- 9.1.5.1 Recommendations by World Organisation for Animal Health / Office of International des Epizooties (OIE) should be followed when packing materials containing infectious agents of animals and birds.
- 9.1.5.2 Screw-capped bottles should be used and should be sealed with adhesive tape or paraffin wax.
- 9.1.5.3 Materials in individually identified containers should be placed in larger strong outer containers and packed with enough absorbent material to protect from damage.

9.1.6 Transportation of Laboratory Animals Regulated by AVA

- 9.1.6.1 Animals should be transported in crates or boxes that must be escape-proof and should have adequate nesting or bedding material where appropriate.
- 9.1.6.2 Transport by air must be in accordance with IATA's Live Animal Regulations.

9.1.7 Transportation of Genetically Modified Organisms (GMOs) and/or GMO-Derived Materials, as specified by Genetic Modification Advisory Committee (GMAC)

- 9.1.7.1 GMAC risk classification is categorized into Category A, B and C, and corresponds to GM materials that are likely to be infectious, low probability of being infectious, and non-infectious, respectively.
- 9.1.7.2 In general, GMOs and GMO-derived materials of Categories A and B must be triple packaged in sealed and unbreakable containers or bags.
- 9.1.7.3 GMOs and GMO-derived materials for air transport should be packaged, marked and labelled according to instructions specified in IATA Dangerous Goods Regulations.
- 9.1.7.4 Transport of Genetically Modified Microorganisms that falls under the purview of BATA, should comply with the regulations specified in BATA 2005 and BATA (Transportation) Regulations 2005.
- 9.1.7.5 Transport of Transgenic Animals should adhere to the National Advisory Committee for Laboratory Animal Research (NACLAR) Guidelines on the Care and Use of Animals for Scientific Purposes.
- 9.1.7.6 Transport of Transgenic Arthropod and their pathogens should adhere to IATA Live Animals Regulation, and the transgenic arthropod and their pathogens should be packaged in triple packaging,
- 9.1.7.7 Transgenic Plants should be transported in a primary container which is packed in a secondary unbreakable container.

9.1.8 International Transport of Infectious Substances and Toxins Specified by International Air Transport Association (IATA)

- 9.1.8.1 International transportation of dangerous goods is regulated by the [International Air Transport Association \(IATA\)](#). The IATA has published the "Dangerous Goods Regulations" (DGR)

which is a manual based on the International Civil Aviation Organization (ICAO) Technical Instructions to provide procedures by which hazardous articles or substances can be safely transported on air. Classification of Dangerous Goods is listed in **Appendix C.3**.

- 9.1.8.2 IATA Regulations forbid dangerous goods (including those in Excepted Quantities) being taken aboard aircraft as hand luggage, checked-in baggage or upon the person. They must always be consigned via a courier.
- 9.1.8.3 Transport regulations and requirements are continually being updated by carriers and government agencies. It is therefore recommended that the Principal Investigator check with all relevant agencies before shipping any regulated material.
- 9.1.8.4 The regulations of the importing country, which may involve applying for permits from their safety, health and environmental agencies, are also to be complied with.
- 9.1.8.5 Definitions of infectious substances, biological products, cultures, patient specimens, medical or clinical wastes, genetically modified microorganisms and organisms, are provided in **Appendix C.1**.
- 9.1.8.6 **Classifications**

For transport purposes, the infectious substances are classified as either Category A or Category B according to IATA Dangerous Goods Regulations (DGR). There is no direct relationship between Risk Groups and categories A and B.

- a. **Category A Infectious Substances** are infectious substances in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. They are assigned the following UN numbers and proper shipping names:
 - UN 2814 – Infectious substance, affecting humans; or
 - UN 2900 – Infectious substance, affecting animals *only*.

Clinical wastes containing Category A infectious substances must be assigned to UN 2814 or UN 2900, as appropriate.

Please refer to the indicative examples in [IATA DGR Table 3.6.D](#) provided in the DGR to assist in the assignment of an infectious substance into Category A, which is not exhaustive. Infectious substances, including new or emerging pathogens, which do not appear in the table but which meet the same criteria must be assigned to Category A.

- b. **Category B Biological Substances** are Infectious Substances that do not meet the criteria for inclusion in Category A. They are assigned the following UN number and proper shipping name:
 - UN 3373 – Biological substance, category B

Clinical wastes containing Category B infectious substances must be assigned to UN 3291.

- c. **Patient Specimen: Exempt Human Specimen or Exempt Animal Specimen**

Patient specimens for which there is minimal likelihood that pathogens are present are not subject to the DGR if the specimen is transported in Packaging for Exempt Patient Specimens.

In determining whether a patient specimen has a minimal likelihood that pathogens are present, an element of professional judgment is required to determine if a substance is exempt under this paragraph. That judgment should be based on the known medical history, symptoms and individual circumstances of the source, human or animal, and endemic local conditions.

Examples of specimens which may be transported under the exemption include the blood or urine tests to monitor cholesterol levels, blood glucose levels, hormone levels, PSA tests, tests required to monitor organ function such as heart, liver, or kidney function for humans or animals with non-infectious diseases, or therapeutic drug monitoring; tests conducted for insurance or employment purposes, biopsies to detect cancer; and antibody detection in humans or animals.

d. Toxins

Toxins from plant, animal or bacterial sources which do not contain any infectious substances or toxins that are not contained in substances which are infectious substances should be considered for classification in Division 6.1, and assigned to the following UN number and proper shipping name:

- UN 3172 – Toxins, extracted from living sources, liquid, n.o.s.
- UN 3462 – Toxins, extracted from living sources, solid, n.o.s.

Toxins from plant, animal or bacterial sources which contain infectious substances, or toxins that are contained in infectious substances, shall be classified in Division 6.2, and assigned to UN2814, UN2900, or UN3373, as appropriate. A toxin known or suspected to contain a Category B infectious substance must be marked UN3373.

e. Genetically Modified Microorganisms and Organisms

Genetically modified microorganisms (GMMOs) or genetically modified organisms (GMOs) not meeting the definition of infectious substance are classified in Class 9 (Miscellaneous dangerous substances and articles, including environmentally hazardous substances). They are assigned the following UN number and proper shipping name:

- UN 3245 – Genetically modified microorganisms; *or*
- UN 3245 – Genetically modified organisms

GMMOs and GMOs are not subject to dangerous goods regulations when authorized for use by the competent authorities of the countries of origin, transit and destination.

Genetically modified live animals shall be transported under terms and conditions of the competent authorities of the countries of origin and destination.

However, if GMMOs and GMOs meet the definition of a toxic substance or infectious substance and the criteria for inclusion in Division 6.1 or 6.2, the requirements for transporting as toxic substance or infectious substance shall apply.

9.1.8.6 Packaging

- a. The basic system of packaging for all infectious substances is triple packaging, which consists

of three layers as follows:

- i. Primary receptacle. A primary watertight, leak-proof receptacle containing the specimen. The receptacle is packaged with enough absorbent material to absorb all fluid in case of breakage.
- ii. Secondary packaging. A second durable, watertight, leak-proof packaging to enclose and protect the primary receptacle(s). Several cushioned primary receptacles may be placed in one secondary packaging, but sufficient additional absorbent material shall be used to absorb all fluid in case of breakage.
- iii. Outer packaging. Secondary packaging is placed in outer shipping packaging with suitable cushioning material. Outer packaging protects their contents from outside influences, such as physical damage, while in transit. The smallest overall external dimension shall be 10 x10 cm.

Absorbent materials may be high performance absorbent pouch, paper towels, vermiculite, disposable diapers, miracle polymers, etc. Ensure there is enough absorbent to contain the entire sample should a leak occur.

b. Packaging Provisions for Infectious Substances, humans or animals, UN 2814 or UN 2900

Packing Instruction 620 in the DGR specifies the type of packaging required for all Category A infectious substances:

- Inner packaging shall comprise:
 - i. leak-proof primary receptacle(s);
 - ii. a leak-proof secondary packaging;
 - iii. other than for solid infectious substances, an absorbent material in sufficient quantity to absorb the entire contents placed between the primary receptacle(s) and the secondary packaging; if multiple fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated so as to prevent contact between them;
- A rigid outer packaging. The smallest external dimension shall be not less than 10 cm (4 in).

All other applicable provisions of the DGR apply. A few selected additional requirements are listed below:

- i. Leak-proof seal shall be provided, e.g. a heat seal, a skirted stopper or a metal crimp seal. If screw caps are used, they shall be secured by positive means, e.g. tape, paraffin sealing tape or manufactured locking closure.
- ii. If ice, dry ice or other refrigerant is used, it shall be placed around the secondary packaging(s) or alternatively in an overpack. If dry ice is used, the outer packaging or overpack shall permit the release of carbon dioxide gas.
- iii. The primary receptacle or the secondary packaging shall be capable of withstanding a pressure differential of not less than 95 kPa.

An example of package containing category A infectious substances is depicted in **Appendix 9.4**.

For UN 2814 and UN 2900, an itemized list of contents shall be enclosed between the secondary packaging and the outer packaging. When the infectious substance to be transported is unknown, but suspected of meeting the criteria for inclusion in category A and assignment to UN 2814 or UN 2900, the words “suspected Category A infectious substance” shall be shown, in parentheses, following the proper shipping name on the document inside the outer packaging.

c. Packaging Provisions for Biological Substances, Category B, Clinical Specimens, Diagnostic Specimens, UN 3373

Packing Instruction 650 in the DGR provides all the information necessary to prepare and transport Category B infectious substances.

If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them.

For liquid substances:

- The primary receptacle(s) must be leak-proof and must not contain more than 1 L;
- The secondary packaging must be leak-proof;
- Absorbent material must be placed between the primary receptacle and the secondary packaging. The absorbent material, such as cotton wool, must be in sufficient quantity to absorb the entire contents of the primary receptacle(s) so that any release of the liquid substance will not compromise the integrity of the cushioning material or of the outer packaging;
- The primary receptacle or the secondary packaging must be capable of withstanding, without leakage, an internal pressure of 95 kPa in the range of -40°C to 55°C.

For solid substances:

- The primary receptacle(s) must be sift-proof and must not exceed the outer packaging weight limit;
- The secondary packaging must be sift-proof;

An itemized list of contents must be enclosed between the secondary packaging and the outer packaging.

At least one surface of the outer packaging must have a minimum dimension of 10 cm × 10 cm (4 in × 4 in).

An example of package containing category B infectious substances is depicted in **Appendix C.4**.

d. Packaging for Exempt Patient Specimens

Packaging for Exempt Patient Specimens shall follow the basic triple packaging outlined in 9.1.8.6.a.

Packaging must be marked "Exempt human specimen" or "Exempt animal specimen", as appropriate. An example of package containing category B infectious substances is depicted in **Appendix C.4**.

If other dangerous goods are present with patient specimens, the relevant provisions of the DGR apply to those goods.

e. Packaging for Toxins

Toxins from plant, animal or bacterial sources which do not contain any infectious substances or toxins that are not contained in substances which are infectious substances, which are assigned to UN 3172 for liquid or UN 3462 for solid, shall be shipped following Packaging Instructions 652 (I), 654 (II), 655 (III) for liquids, and 666 (I), 669 (II) or 670 (III) for solids for shipment on passenger aircraft.

f. Packaging for GMMOs and GMOs

GMMOs and GMOs not meeting the definition of infectious substance are classified in Class 9 (Miscellaneous dangerous substances and articles, including environmentally hazardous substances) and assigned to UN 3245 shall be shipped following Packaging Instruction 954. GMMOs and GMOs packed and marked according to Packaging Instruction 954 are not subject to any other requirements in the DGR.

9.1.8.7 Marking, Labelling & Documentation

a. Marking

Marking on each package shall display the following information on the outer packaging or the overpack:

- i. the shipper's (sender's, consignor's) name and address
- ii. the telephone number of a responsible person, knowledgeable about the shipment
- iii. the receiver's (consignee's) name and address
- iv. the United Nations number followed by the proper shipping name:
 - **Category A Infectious Substances:** UN 2814 "Infectious substance, affecting humans" or UN 2900 "Infectious substance, affecting animals *only*", as appropriate
 - **Category B Infectious Substances:** UN3373 "Biological substance, category B" adjacent to the diamond-shaped mark "UN3373" shown in **Appendix C.5**
- v. temperature storage requirements (optional)
- vi. when dry ice or liquid nitrogen is used: the technical name of the refrigerant, the appropriate United Nations number, and the net quantity

b. Labelling

Orientation labels, hazard labels and handling labels shall be affixed on the outside of each package for all dangerous goods to be shipped unless exempted. Some applicable labels are shown in **Appendix C.5**, including:

- i. Orientation label, "This End Up" or "This Side Up", to illustrate correct vertical orientation

- ii. Hazard label for Toxins (Class 6)
- iii. Hazard label for Category A infectious substances (Class 6)
- iv. Diamond-shaped mark for Category B infectious substances
- v. Hazard label for Miscellaneous Dangerous Goods, including hazard label for dry ice (Class 9)

Shipping information for air transport of various biological materials is summarized in **Appendix C.6**.

c. Documentation

Documentation to be prepared and signed by shipper for Category A Infectious Substances:

- i. the shipper's Declaration for Dangerous Goods (please refer to an example of Shipper's Declaration for Dangerous Goods and instruction guide in **Appendix C.7**)
- ii. packing list / proforma invoice that includes the receiver's address, the number of packages, detail of contents, weight, value)
- iii. an import and/or export permit and/or declaration if required

Documentations to be prepared and signed by the shipper for Category B Infectious Substances:

- i. for international shipments: a packing list / proforma invoice that includes the shipper's and the receiver's address, the number of packages, detail of contents, weight, value
- ii. an import and/or export permit and/or declaration if required

Dangerous goods documentation (including a shipper's declaration) is not required for Category B infectious substances.

9.1.7 Training

Training is mandatory for those involved in the packaging or transport of biological agents.

9.1.8 Records

Records of the following are to be kept for 3 years:

- a. The facility / institution's biosafety and security plan
- b. Current list of individuals with access to select agents for purpose of transfer/transport
- c. Particulars of individuals transferring and receiving biological agents
- d. Training records for individuals with access to select agents for purpose of transfer / transport
- e. Accurate and current inventory records for laboratory / facility performing transfer / transport
- f. Permits and transfer documents
- g. Security records (e.g. transactions from automated access control systems, testing and maintenance of security systems, visitor logs)
- h. Biosafety, containment, and security incident reports

9.2 TRANSPORT OF NON-REGULATED BIOLOGICAL MATERIALS

These guidelines details how biological materials that are not regulated under the Biological Agents & Toxins Act and Animals and Birds Act, and exempted in Singapore Biosafety Guidelines for Research on Genetically Modified Organisms, are to be safely transported between laboratories within the same building, between National University of Singapore (NUS) buildings, into and out of NUS. This is to ensure that the public and the workers in the transportation chain are protected from exposure to any substances that might be in the package.

All biological materials applicable to this SOP are for research purposes only.

9.2.1 Scope

- a. This procedure covers the transport of biological materials within, into or out of NUS, for research purposes only.
- b. All NUS staff and students are to follow these procedures.
- c. Researchers and visitors from other institutions/organizations who have collaborations with NUS are to follow these procedures when transporting/transferring biological materials within NUS.
- d. This procedure covers the transport of non-regulated biological materials not exceeding 500 milliliters in volume or 500 grams in weight.

9.2.2 Responsibilities

- a. Principal Investigator
The PI is to ensure that all staff and students under his/her supervision follow this SOP for the transportation of biological materials.
- b. Transferor
The transferor can be research staff or students assigned by the PI for transporting the biological materials to an intended destination. He/she should ensure that the packaging and labeling of container as well as other transportation procedures are in accordance with those described in this SOP.
- c. Transferee
All NUS staff or students should ensure that the package is intact and its contents are accounted for upon receipt of the biological materials.

9.2.3 Definitions

- a. In this procedure, transport refers to the movement of biological materials between laboratories within the same building, between buildings within NUS, into and out of NUS.

- b. Biological materials are referred to as substances deriving from living sources (such as humans, animals, plants, and microorganisms). The sources of human material could be from subjects following diagnostic or therapeutic procedures, autopsy specimens, donation of organs or tissue from living or dead persons, fetal tissue, body wastes or abandoned tissue. These materials are classified as follows:
 - i. Human/animal materials, which may include but are not limited to, organs and parts of organs; cells and tissue including commercially available cell lines; sub-cellular structures and cellular products: blood; gametes (sperm and ova); embryos and fetal tissue.
 - ii. Human waste such as urine, faeces, sweat, hair, epithelial scales, nail clippings, placenta, saliva / sputum etc.
- c. Biological materials that are excluded in this manual (but for which similar packaging is recommended) are:
 - i. Materials with a low probability of containing an infectious agent or where the concentration of the infectious substance is at a level which is naturally occurring in the environment, whereby it is unlikely to cause disease when exposed to it, e.g. foodstuffs and environmental samples such as water or a sample of dust or mold.
 - ii. Adequately fixed (using formaldehyde or glutaraldehyde) tissue blocks whereby the biohazard has been eliminated. However, cryofixed / frozen specimens are included in the scope of this SOP.
 - iii. All genomic/recombinant DNA, RNA and siRNAs.
- d. Lentiviral vectors and vectors derived from agents that are regulated under BATA, shall follow the guidelines outlined in Transport of Regulated Biological Materials.

9.2.4 Procedure for Packaging

- a. The sample must be triple packaged (please see example in **Appendix C.8**). The sample must be placed in a sealed primary container that is securely closed, and leak-proof. Plastic containers should be used whenever possible.
- b. The primary container should be placed in a leak-proof and sealed watertight secondary container, with enough absorbent material (e.g. paper towels or commercially-available absorbent material) placed around the primary container sufficient to absorb the entire contents of the primary container in case of breakage or leakage.
- c. If the primary container is fragile, it must be individually wrapped or separated to prevent contact between multiple primary containers.
- d. If the outside of the primary container is suspected of being contaminated, decontaminate, prior to placing it into the secondary container, using a disinfectant appropriate for the biological material, e.g. 10,000 ppm hypochlorite, or any other efficacious registered disinfectant (for example, registered with U.S Environmental Protection Agency).

- e. Information sheet including quantity and type of biological material, and particulars about the transferor and transferee (including emergency contact numbers) should be pasted on the secondary container.
- f. The secondary container must be placed in a tertiary carrier/cooler that is sturdy and leak-proof, with a lid that can be fastened.
- g. The outside of the tertiary container must be free of any biohazardous material so that the package can be carried safely between facilities or outside NUS without wearing gloves or lab coats.
- h. It is good practice to carry a spill kit containing absorbent pad, gloves, disposable labcoat, and appropriate disinfectant during the transport. Do not attempt to clean it up without appropriate spill response material. Keep other persons away from the spill.

9.2.5 Procedure for Transporting

- a. Biological materials may be transported between laboratories within the same building using an enclosed secondary containment such as a cooler box with sufficient absorbent material enough to contain the entire content of the primary container.
- b. Biological materials can be transported via walking to the destination within NUS campus.
- c. A commercial courier, University vehicle, personal vehicle or taxi can be used for transport within or outside NUS campus.
- d. Motorcycles may be used, provided that triple packaging procedure has been followed and the package is secured in the motorcycle's case.
- e. Public buses and trains are not allowed for transporting the said biological materials.
- f. The package must be taken directly to its intended location without stopping at other locations along the way and shall not be opened during transport. If a spill is suspected, the package shall be opened at the destination inside a Biological Safety Cabinet.
- g. Please contact OSHE for contact information of some local couriers and commercial packaging materials.

9.2.6 Safety Precaution

- a. The use of plastic container instead of glassware is strongly recommended to minimize the potential risk of injury in the case of breakage during handling.
- b. Wipe down the containers with appropriate disinfectant to eliminate any contaminants.
- c. The transferor must follow this SOP for proper packing and labeling of the material in the container.

CHAPTER 10 COMMON CHEMICALS AND RADIOACTIVE MATERIALS

This chapter summarizes the safe handling of common laboratory chemicals as well as radioactive materials often used in biomedical research laboratories. Please refer to [NUS Laboratory Chemical Safety Manual](#) and [NUS Laboratory Ionizing Radiation Safety Manual](#) for more detailed information.

10.1 LIQUID NITROGEN

Liquid nitrogen is the liquid state of nitrogen at an extremely low temperature. At atmospheric pressure, liquid nitrogen boils at -196°C (77 K; -321°F) and is a cryogenic fluid which can cause rapid freezing on contact with living tissue. It has the ability to maintain temperatures far below the freezing point of water, which makes it extremely useful in a wide range of applications, including:

- the storage of cells at low temperature for laboratory work
- in cryogenics
- for the cryopreservation of blood, reproductive cells (sperm and egg), and other biological samples and materials
- the preservation of tissue samples from surgical excisions for future studies.

Because of its extremely low temperature, careless handling of liquid nitrogen may cause cold burns. As liquid nitrogen evaporates it will reduce the oxygen concentration in the air and may act as an asphyxiant, especially in confined spaces.

10.1.1 Handling

- a. Use leather or thermally insulated gloves for handling cold materials and cryogenic liquids (e.g. liquid nitrogen). When handling cryogenic liquids (e.g. liquid nitrogen), the gloves should be impervious and sufficiently large to be readily removed should a cryogen be spilled. Watches, rings, and other jewellery shall be removed before the user puts on the glove.
- b. Unprotected body parts shall not come in contact with vessels or pipes that contain cryogenic liquid because extremely cold material may stick firmly to the skin and tear flesh if separation is attempted. To provide adequate bodily protection, wear an impervious apron / coat over a long sleeve shirt and long pants and use tongs to handle objects that are in contact with the cryogenic liquid.
- c. Wear closed-toe shoes and ensure the end of the pants is placed over the shoe/ boot tops to prevent shoes filling with liquid nitrogen in the event of a cryogenic liquid spillage.
- d. Wear a face shield to protect the eyes and other parts of the face from the splashes of liquid nitrogen.
- e. All equipment should be kept clean.
- f. Work areas should be well ventilated.
- g. Transfer or pouring of cryogenic liquid should be done very slowly to minimize boiling and splashing. Transfer of liquid nitrogen should be done in a well-ventilated area.
- h. Cryogenic liquids and dry ice used as refrigerant baths should be open to the atmosphere. They should never be in a closed system where they may develop uncontrolled or dangerously high pressure.

10.1.2 Storage

- a. Liquid nitrogen containing equipment or containers should be stored in a well-ventilated area with tiled flooring instead of vinyl flooring to prevent damage to the floor.
- b. Cryogenic liquids should be handled and stored in containers that are designed for the pressure and temperature to which they may be subjected. The most common container for cryogenic liquids is a double-walled, evacuated container known as a Dewar flask.
- c. Containers and systems containing cryogenic liquids should have pressure relief mechanisms.
- d. Cylinders and other pressure vessels such as Dewar flasks used for the storage of cryogenic liquids should not be filled more than 80% of capacity, to protect against possible thermal expansion of the contents and bursting of the vessel by hydrostatic pressure. In case the temperature of the cylinder may increase to above 30°C (86°F), it is highly recommended that a lower percentage (i.e. 60 percent capacity) should be the fill limit.
- e. Dewar flasks should be shielded with tape or wire mesh to minimize flying glass fragments should an implosion occur. Dewar flasks should be labeled with the full cryogenic liquid name and hazard warning information.
- f. Transportation of cryogenic liquids via elevators should be unmanned and a buddy system adopted, where one person pushes the cryogenic liquid tanks inside the lift at one level and the other person waits at the destination floor to receive the tanks.

10.1.3 Training

All staff using or handling cryogenic liquids must receive training that includes hazards associated with its use, care, selection and use of protective equipment and emergency procedures. New users of liquid nitrogen should receive instruction and oversight in its use from experienced members of the academic or technical staff.

10.1.4 First Aid

- a. Warm the affected area of the body rapidly by immersion in water not to exceed 40°C, with body heat, or by exposure to warm air. In the event of massive exposure, the emergency shower should be used to warm the body. All clothing must be removed prior to showering. Maintain the affected area of the victim at normal body temperature until medical help arrives.
- b. Calm the victim and prevent aggravation of the injury. People with frostbitten feet should not walk on them. Do not rub or massage the affected parts of the body.
- c. Prevent infection – use a mild soap to clean the affected area. Dressings need to be applied if the skin is intact.
- d. Flush eyes, if affected, with warm water for 15 minutes.

10.2 DNA CHELATORS

Ethidium bromide (EtBr) is commonly used to stain for the visualization of nucleic acids in gel electrophoresis and other methods of nucleic acid separation. EtBr appears as a dark red, crystalline, non-volatile solid, moderately soluble in water. It is available commercially as a 10 mg/ml solution. The stain fluoresces readily with a reddish-brown color when exposed to ultraviolet (UV) light. It is a powerful mutagen as it intercalates DNA; is moderately toxic on acute exposure by ingestion, inhalation or absorption through the skin; and is an irritant to the skin, eyes, mouth, and upper respiratory tract.

There are safer alternatives to EtBr in the market that help to reduce potential exposure to the hazard. Examples are SYBR, SYBR Safe™, SYBR GOLD, GelRed, GelGreen, FluroSafe and Atlas Cleansight stains. These are less mutagenic and less toxic.

10.2.1 Safe Handling of Ethidium Bromide

- a. Purchase ready-made solutions of EtBr so as to avoid the higher risk of inhalation of the powder form. Powdered EtBr can also be difficult to clean up safely if spilled. Otherwise, work with EtBr powder or crystals in a fume hood to prevent inhalation exposure.
- b. To minimize your exposure to EtBr, purchase pre-mixed dilute solutions of EtBr in the smallest practical quantity. This avoids exposure to concentrated EtBr during mixing.
- c. Label all designated areas or equipment that come in contact with EtBr with appropriate warning signs.
- d. It is recommended that an absorbent bench liner or underpad with waterproof backing be used on the bench area designated for EtBr usage. The absorbent surface liner should be replaced regularly or when contaminated.
- e. Work surfaces should be washed/wiped and disinfected at the end of an experiment, at the close of the day, and after a spill.
- f. Wear a lab coat, closed-toe shoes, chemical resistant nitrile gloves and goggles when handling EtBr. Change/discard your gloves after handling EtBr.
- g. Always wash hands thoroughly after handling EtBr, even if gloves are used.
- h. Ensure that there is unobstructed access to an eyewash/shower unit in the work area.
- i. Store in a secondary container to prevent surface contamination in case of spillage / leakage.
- j. Wear UV-blocking eyewear or work behind a UV shielding glass when using ultraviolet light to visualize EtBr.
- k. In the event of a spill, the recommended procedures are as follows:
 - **Contact with the eyes:** Immediately flush with large amounts of water for at least 15 minutes, preferably with the use of an emergency eyewash.
 - **Contact with skin:** Remove contaminated clothing/gloves and immediately wash affected area with soap and large amounts of water for 15 minutes.
 - **If swallowed or inhaled:** If swallowed, obtain medical attention immediately. If EtBr dust is inhaled, move the affected person to an area with fresh air.
 - After any exposure to EtBr or all other DNA chelators (via skin, inhalation, ingestion, or eye contact), report to [NUS Accident / Incident Reporting System \(AIRS\)](#) and immediately seek a medical attention from the University Health Centre (UHC).
 - Always wear protective equipment available in the chemical spill kit such as face/eye protection, chemical resistant gloves (e.g. nitrile for EtBr), splash resistant gown and chemical resistant boots.
 - Use a spill pillow or absorbent material to soak up liquid EtBr. Carefully clean up solid (powder/crystal) EtBr to avoid creating dusts. Dispose in a waste container designated for EtBr. For spills of powdered EtBr, carefully wipe it up with wet paper towels
 - Use a UV light source to illuminate the area to ensure that there is no remaining EtBr.
 - Dispose of all contaminated items (gloves, paper towels, spill cleanup materials) into labeled, double bags or waste container designated as cytotoxic waste.
- l. EtBr wastes are classified as cytotoxic waste and should be disposed off via a licensed waste collector for special medical waste incineration.

- *Solid wastes:* Agarose gels containing EtBr should be discarded in **purple cytotoxic leak-proof plastic bags**. Items such as gloves, pipette tips, paper towels, etc. that are grossly contaminated with EtBr should be discarded similarly. The wastes should then be disposed via a licensed waste collector.



Figure 10.1 Purple Cytotoxic Waste Bag

- *Solutions:* Aqueous solutions containing $\leq 1 \mu\text{g/ml}$ (1 ppm) EtBr can be discharged into the lab sink. Solutions containing $>1 \mu\text{g/ml}$ (1 ppm) EtBr shall be decontaminated using one of the methods described below.
- m. **Decontamination** of large volumes of EtBr solutions can be accomplished by filtration or adsorption methods. Chemical inactivation by bleach is not recommended due to its poor efficacy and the production of more mutagenic compounds. Other methods could potentially introduce other chemical hazards and are also discouraged.
- *Filtration:* Filter EtBr waste solutions through a bed of activated charcoal. Many commercial filter funnel kits with packaged charcoal are available (e.g. Schleicher & Schuell Extractor® Ethidium Bromide Waste Reduction System, Sigma Extractor for Ethidium Bromide Decontamination). Follow the product instructions for proper use. EtBr is bound to the charcoal filters which can be disposed of as solid cytotoxic waste. The filtrate may be poured down the drainage system.



Figure 10.2 Filtration System for Ethidium Bromide Decontamination

- *Absorption:* Use absorbent material (e.g. Qbiogene EtBr GreenBag™ Disposal Kit, Amresco Destaining Bags) containing activated carbon which absorbs the EtBr in solution. Incubate the bags in the solution according to the product instructions noting the absorbent capacity per bag with the total amount of EtBr in the buffer. Discard the bags containing adsorbed EtBr as solid EtBr waste. The treated buffer may be poured down the drainage system.



Figure 10.3 Absorption System for Ethidium Bromide Decontamination

10.2.2 Safe Handling of Ethidium Bromide Alternatives

- As there are insufficient data on the long-term effects of EtBr alternatives, such DNA chelators are to be handled using procedures similar to the use of EtBr as described in Section 10.2.1.
- Solid wastes:** Agarose gels containing non-EtBr alternatives, including contaminated lab debris e.g. gloves, pads, towels and tubes, shall be discarded in **purple cytotoxic leak-proof plastic bags**.
- Liquid wastes** e.g. electrophoresis buffers, staining solutions, etc. shall be discarded as cytotoxic waste.

10.3 TOXINS

Biological toxins are typically poisons of natural origin or are manufactured synthetically and may cause severe incapacitation and even death at low exposure levels. Typical toxins can range from bacterial origin such as cholera toxin to snake venoms.

Please refer to Section 3.3 for details on the regulations and biosafety requirements for toxins under the Fifth Schedule of the BATA. These agents include *Botulinum* toxins (types A, B, C, D, E, F and G), *Clostridium perfringens* toxins, *Staphylococcal enterotoxin* B, Shigatoxins, T-2 toxin, Tetanus toxins, Verotoxins and HT-2 toxin.

10.3.1 Safe Handling of Biological Toxins

- Users shall ensure the responsible use and security of toxins by storing them in a lockable cabinet with an active inventory where each use is recorded and accounted for.
- Toxins can generally be handled using guidelines for hazardous chemicals with special attention to accidental exposure routes by direct contact with the mouth, eyes or other mucous membranes; through generation of aerosols and by needles or other hazards that can penetrate the skin.
- As with any procedure, a risk assessment shall be conducted before starting work and supervised practice runs are rehearsed without the active toxin to familiarize researchers with the overall procedures as well as the response to emergency situations such as a spillage.

- d. Preparation of toxins poses the greatest risk of occupational exposure due to the initial concentration and quantities used. This includes preparing a pre-measured single dose unit (e.g. drawing up toxins in liquid form from a vial into a syringe) and emptying capsules to prepare partial doses. For lyophilized samples, it is recommended to order the smallest quantity and then make up a stock solution directly from the vial without additional weighing.
- e. The principal focus of safety during preparation of poisons should be on operator protection, product protection (maintenance of product sterility and stability), protection of the working environment and protection of the end user.
- f. Engineering controls such as dedicated fume hoods and exhaust systems that do not allow air to re-circulate back into the room can be used or some means of isolating or enclosing the materials in a suitable fashion so that untrained or unaware persons are not accidentally exposed to the compounds.
- g. Laboratory work with toxins is to be performed in dedicated rooms with controlled access and a pre-determined bench area. When toxins are in use, a warning sign should be posted. Visitors or other untrained personnel are to be monitored and protected from handling equipment used in the manipulation of the toxin.
- h. It is recommended that routine operations with dilute toxin solutions be performed in a BSL2 containment facility within a well-maintained biological safety cabinet. Low molecular weight toxins or work involving volatile chemicals or radionuclides combined with toxin solutions may require a charcoal-based hood filter in addition to the HEPA filter.
- i. Laboratory coats shall be long-sleeved with an elastic band such that the hands and arms are completely covered when worn together with an appropriate pair of gloves. The choice of gloves and other protective clothing would depend on the nature of the toxin as well as the choice of solvent used.
- j. Toxin stocks shall be removed from the biological safety cabinet only after the exterior surface of the closed primary container has been decontaminated and placed in a clean secondary container. Toxin stocks shall be transported in leak-proof secondary containers within the laboratory and the interior of the biological safety cabinet shall be thoroughly decontaminated after use.
- k. Centrifugation of toxins shall be carried out in sealed rotors or rotors with safety cups and the outer surface of the centrifuge tubes shall be cleaned before centrifugation to prevent the inadvertent generation of aerosols. After centrifugation, the entire rotor is to be taken into the biological safety cabinet to open it and remove the sample tubes.
- l. The use of glassware and sharps shall be avoided especially when working at lethal doses. Only experienced animal handlers shall inoculate animals using hollow-bore needles and the sharps bin shall be decontaminated as soon as practicable.
- m. Contaminated materials and toxins may be inactivated by incineration or by soaking in suitable decontamination solutions (**Appendix D.1**).
- n. In case of spills, avoid splashes or generating aerosols during the cleanup process by using sufficient absorbent material to overlay the spill. A suitable decontamination solution shall be applied from the perimeter towards the center of the covered spill and be allowed sufficient contact time to completely inactivate the spill (**Appendix D.1**).
- o. The Safety Data Sheet shall be kept and provided to a physician to assist in treatment. Where applicable, antidotes, especially for snake venoms, are to be made available and administered by competent personnel.

10.4 CYTOTOXIC DRUGS

Cytotoxic drugs can be defined as drugs or enzymes that are administered to plants, animals or humans for the specific purpose of altering the metabolic process or possessing a specific destructive action on

cells. They may be genotoxic, oncogenic, mutagenic, and teratogenic and include most anti-cancer drugs. Common laboratory cytotoxic drugs include cyclophosphamide, methotrexate, bleomycin, etoposide, etc.

Contact with cytotoxic drugs can cause immediate problems, such as dermatitis, dizziness, nausea and headache. Studies suggest that repeated exposure to small amounts of the drugs may cause organ or chromosome damage or impaired fertility. The carcinogenic, teratogenic and mutagenic nature of cancer chemotherapeutic agents has been well documented in animal models and at therapeutic levels in patients.

Handling of cytotoxic drugs requires medical surveillance as they are carcinogens which affect the bone marrow, liver, kidneys and reproductive system (in addition to other organ systems).

Cytotoxic drugs listed under the [Poisons Act](#) and administered under the [Health Sciences Authority of Singapore \(HSA\)](#) where the relevant responsibilities of researchers in NUS include:

- a. Imported poisons require an import permit from HSA while poisons ordered from local vendors are excluded.
- b. Poisons must be kept under lock and key in a designated poison cupboard.
- c. Maintain and update inventory with a check every six months
- d. Maintain, update and make available Safety Data Sheets (SDS) and ensure the validity of SDS to within 5 years.
- e. Proper labeling of the scheduled poison with a distinction or mark indicating that it contains poison.
- f. Liquid chemicals must be stored within a secondary containment tray that can capture 20% of the total chemicals stored in the tray.
- g. Disposed through a toxic industrial waste collector.

10.4.1 Safe Handling of Cytotoxic Drugs

- a. As with any chemical agent, a risk assessment has to be performed and the hazards addressed with the proper hierarchy of control measures where elimination, substitution, engineering control, administrative control and finally PPE is considered to mitigate the exposure to the researchers.
- b. Preparation of poisons, poses the greatest risk of occupational exposure due to the initial concentration and quantities used. This includes preparing a pre-measured single dose unit (e.g. drawing up cytotoxic drugs in liquid form from a vial into a syringe) and crushing or dissolving tablets or emptying capsules to prepare part doses.
- c. The principal focus of safety during preparation of poisons should be on operator protection, product protection (maintenance of product sterility and stability), protection of the working environment and protection of the end user.
- d. Engineering controls such as dedicated fume hoods and exhaust systems that do not allow air to re-circulate back into the room can be used or some means of isolating or enclosing the materials in a suitable fashion so that untrained or unaware persons are not accidentally exposed to the compounds.
- e. Administratively, training and competency assessments are crucial. In addition, if such compounds are shipped lyophilized, multiple aliquots can be made from the same vial so as to avoid weighing the powders and to reduce the potential of spreading airborne particles once they are in solution. If there is a need to weigh, do so with an enclosed balance in the fume hood. It would also be prudent to order the required amount per use and not stockpile such

compounds just in case of an unforeseen crack or spill where a lesser volume would be more manageable to contain and clean up.

- f. For PPE, the choice of gloves depends on the chemical nature of the compound as well as the solvent used. A disposable long-sleeved gown made of lint-free fabric with knitted cuffs and a closed front is recommended to be worn during the preparation of cytotoxic drugs. This is to prevent any potential powder and splashes to be carried onto the main laboratory coat.
- g. Spills outside the containment equipment have to be managed using a chemical spill kit with eye protection and an appropriate respirator if powdered compounds are spilled. Cytotoxic drugs are to be disposed as cytotoxic waste via a toxic industrial waste collector. As with all incidents, do report it to your supervisor and to [NUS Accident / Incident Reporting System \(AIRS\)](#).
- h. Cytotoxic drugs are typically used in varying dilutions for a particular period of incubation time where the medium will be changed. The spent medium collected may be treated with an absorbent material such as vermiculite and disposed as solid cytotoxic waste. Where the volume of cytotoxic wastes are large and the use of absorbent materials deemed not practical, there has been some data using chemical inactivation such as potassium permanganate and sodium hypochlorite on some common cytotoxic drugs (**Appendix D.2 and Appendix D.3**). If there is any uncertainty, cytotoxic drugs are to be disposed via licensed Toxic Industrial Waste (TIW) contractors.

10.5 POISONS

This section describes the general safe work practices when handling chemicals marked with the poisons label that may or may not be accompanied by the typical skulls and crossbones picture. The scope of this section is limited to general chemicals. For poisons regulated by the [Health Sciences Authority \(HSA\)](#) of Singapore, please refer to the relevant sections on toxins, cytotoxic drugs or antibiotics for more information.

10.5.1 Safe Handling of Non-HSA Regulated Poisons

- a. Personnel shall be familiar with the Safety Data Sheets on the hazards and health risks via common routes of exposure due to the nature of their work processes. When handling large quantities of poisons, do consider air monitoring and health surveillance. For this purpose, a [Semi-Quantitative Risk Assessment for Chemical Exposure Workbook](#) would be helpful.
- b. Competent personnel can only perform such procedures with permission from the PI and suitable equipment such as centrifuges with safety caps must be used to prevent accidental exposure. Do avoid sharps to prevent accidental subcutaneous inoculations and cover work surfaces with an absorbent bench pad that is to be replaced regularly and after a spill. Handle powders inside a certified chemical fume hood to minimize exposure to dust.
- c. Personal protective equipment shall be non-porous, including the laboratory coats and safety glasses. The choice of gloves shall also depend on the solvent used for the poison. Appropriate respirators and isolation of the work area shall be implemented if the process cannot be adequately contained.
- d. Laboratory equipment shall be decontaminated with an appropriate chemical cleanser and workbenches wiped down regularly after work. Decontaminate equipment routinely, especially prior to servicing.
- e. Wastes shall be appropriately labeled and disposed via Toxic Industrial Waste contractors and stored in a segregated area prior to disposal to reduce risk of exposure to personnel.

- f. Spills shall be treated using a chemical spill kit or a poison specific spill kit for example as those for mercury. Cleanup material shall be bagged up and disposed via Toxic Industrial Waste contractors.

10.6 ANTIBIOTICS

Antibiotics are typically used in the biomedical laboratory for the selection of host systems expressing recombinant DNA and as a supplement to cell culture media in most laboratories. The effects of exposure are not as severe as compared to cytotoxic drugs but the effects range from skin sensitization to severe allergic reactions depending on the individual's medical history.

Antibiotic drugs are listed under the [Poisons Act](#) and administered under the [Health Sciences Authority \(HSA\)](#) of Singapore where the relevant responsibilities of researchers in NUS include:

- a. Poisons to be imported require an import permit from HSA while poisons ordered from local vendors are excluded.
- b. Poisons must be kept under lock and key in a designated poison cupboard.
- c. Maintain and update inventory with a check every six months
- d. Maintain, update and make available Safety Data Sheets (SDS) and ensure the validity of SDS to within 5 years.
- e. Proper labeling of the scheduled poison with a distinction or mark indicating that it contains poison.
- f. Liquid chemicals must be stored within a secondary containment tray that can capture 20% of the total chemicals stored in the tray.
- g. Disposed through Toxic Industrial Waste collector.

10.6.1 Safe Handling of Antibiotics

- a. As with any chemical agent, a risk assessment has to be performed and the hazards addressed with the proper hierarchy of control measures where elimination, substitution, engineering control, administrative control and finally PPE is considered to mitigate the exposure to the researchers.
- b. Antibiotics are available as a concentrated stock solution for cell culture use or in a powdered form for molecular biology cloning work. Therefore, the choice of control measures depends on the physical state of the antibiotic.
- c. Concentrated liquid stocks are typically aliquoted into single use supplements in falcon tubes to be frozen down for the next use. This is performed in a biological safety cabinet to maintain sterility and to protect the operator from splashes to the face. Potential exposure would thus be limited by wearing the appropriate gloves as a barrier against skin contact as well as a laboratory coat should there be a large splash.
- d. The weighing of powdered antibiotics constitutes a major hazard to operators due to the generation of airborne powders. This shall be performed in an enclosed balance in the fume hood to minimize the generation of airborne powders.
- e. Spills outside any containment equipment have to be managed using a chemical spill kit with eye protection and an appropriate respirator if powdered antibiotics are spilled. Antibiotics are to be disposed as chemical waste via licensed Toxic Industrial Waste contractors. As with all incidents, do report it to your supervisor and to [NUS Accident / Incident Reporting System \(AIRS\)](#).

- f. The disposal of stock antibiotics is strictly through licensed Toxic Industrial Waste collectors. A few antibiotics may be successfully inactivated via autoclaving or boiling (**Appendix D.4**) and may be used as a reference to treat stock antibiotics in the laboratory. In there is any uncertainty, antibiotics are to be disposed as chemical waste.

10.7 DISINFECTANTS

Disinfectants are chemicals used in laboratories to inactivate or kill microorganisms. To accomplish this, the chemicals have properties such as being an oxidizer, flammable, or an irritant that can be hazardous to humans. The details of the most commonly used disinfectants are discussed in Chapter 8 of this manual. Always read and follow the directions on the label for dilution rates, application method, specific hazards and disposal procedures. See Chapter 8 for more details.

10.8 NANOPARTICLES

Nanotechnology is defined as the manipulation of matter at the atomic, macromolecular levels in the length scale of approximately 1-100 nm range to produce new materials, structures, and devices with unique properties and functions because of its small size. Some of the applications of nanoparticles used for biology are:

- Fluorescent biological labels
- Drug and gene delivery
- Bio detection of pathogens
- Detection of proteins
- Probing of DNA structure
- Tissue engineering
- Tumour destruction via heating (hyperthermia)
- Separation and purification of biological molecules and cells
- MRI contrast enhancement
- Phagokinetic studies

Nanomaterials have the greatest potential to enter the body through the respiratory system should they become airborne and in the form of respirable-sized particles (nanoparticles). They may also come into contact with the skin or be ingested.

Risk assessments shall emphasize on key factors to consider when working with engineered nanoparticles and should include but not be limited to the following: size & size distribution, shape, mass, concentration & numbers, process, properties and location of work.

Engineering control techniques such as source enclosure and local exhaust ventilation systems should be effective for capturing airborne nanoparticles. High-Efficiency Particulate Air (HEPA) filter should effectively remove nanoparticles.

Respirators such as N95 are sufficient for particles as small as 2.5 nm and it is recommended to use N, P or R95 (HEPA) particulate respirators for protection against inhalation exposure

All nanomaterial wastes must be disposed off as chemical waste.

For detailed information on nanoparticles, please refer to Chapter 8 of the [NUS Laboratory Chemical Safety Manual](#) (NUS/OSHE/M/02).

10.9 RADIOACTIVE MATERIALS

The use of radioisotopes in biomedical research laboratories in NUS typically range from molecular biology such as tagging nucleic acids to *in vitro* applications such as metabolic labeling where the synthesis and post-translational modification can be determined. This section provides information regarding regulations and recommended safe procedures to protect the operator and prevent spread of contamination. For more information, please refer to the [NUS Laboratory Ionizing Radiation Safety Manual](#).

10.9.1 Radiation Workers

- a. All individuals working directly with radioactive sources are termed as radiation workers and are required to complete the NUS Ionizing Radiation Safety Training before starting work. They are also required to ensure that the supervisory L6 and radiation worker R1 licenses are valid and the personal dosimeters are exchanged on time.
- b. It is good practice to have the entire protocol planned out and rehearsed advance to minimize potential problems. This would include transporting the hot samples to waste disposal, decontamination and spill procedures.
- c. Personal dosimeters are meant to measure each individual's exposure and shall not be shared under any circumstance.
- d. Inventories shall be securely stored and users are to account for the use of radioisotopes.

10.9.2 Safe Handling of Radioactive Materials

- a. The use of double gloves is encouraged as the user would still be protected by the inner glove should the outer glove require changing in the middle of the procedure.
- b. A laboratory coat, closed-toe shoes and face / eye protection must be worn to protect against splashes.
- c. Radioisotopes that present inhalation hazards such as radioiodine shall only be handled in a dedicated fume hood to minimize personnel and environmental exposure.
- d. Prominent warning signs with the universal radiation logo are to be placed on the workstation and on equipment used for radioactive work. A radioactive warning tape can be used to demarcate the radioactive work area. The work surface should also be covered with absorbent material with an impervious backing to facilitate cleaning after the procedure.
- e. Operators are to work behind a shield that is suitable for the radioisotope. Commercial perspex shields are ideal to protect against beta emitters such as C-14, S-35, P-32, etc. and commercial lead shields are used for gamma emitters such as Cs-137, I-125, Cr-51, etc. Lead shields shall not be used for beta emitters due to the generation of *Bremsstrahlung* X-rays from the lead shield. However, I-131 which emits both gamma and moderate beta emissions can be used with lead shields to control the production of X-rays.
- f. The use of disposable materials is encouraged to minimize the need for decontamination.
- g. Wastes shall be disposed of in a red radiation waste bag located inside a shielded box made of a material that is suitable for the particular radioisotope. Liquid wastes shall be absorbed with a chemically compatible absorbent, as only solid radioactive wastes can be disposed.

- h. Cleaning materials such as absorbent material and detergents are to be placed near the work area. Dispose all clean-up materials into the radioactive waste container and segregate radioactive wastes from different isotopes into different containers.
- i. The workbench and equipment shall be monitored for radioactive contamination before and after the procedure. Such exposure monitoring can be performed by either using an energy compensated GM probe or by using an ionizing chamber. Liquid scintillation count of surface and equipment wipe tests shall be performed periodically. Please refer to the [NUS Laboratory Ionizing Radiation Safety Manual](#) for detailed instructions. Based on such monitoring, users can determine the necessity for subsequent rounds of surface decontamination.
- j. A suitable spill response kit for radioactive is to be placed in the laboratory and all the radiation workers are to be competent in responding to such spills. Report all spills to the PI and submit a report to [NUS Accident / Incident Reporting System \(AIRS\)](#).
- k. Disposals are to be coordinated with OSHE. Please refer to the [NUS Laboratory Ionizing Radiation Safety Manual](#) for details.

CHAPTER 11 PERSONAL PROTECTIVE EQUIPMENT

Personal Protective Equipment (PPE) are often used in combination with biological safety cabinets and other containment equipment to protect personnel from contact with biological agents and toxins, animals, other materials such as toxic and corrosive chemicals, heat, cold, fire and other physical hazards. Appropriate PPE may also protect the experiment from contamination.

The extent and kind of clothing and equipment to be selected for any particular activity depends upon the research operations and levels of risk associated with the research. It should be understood that PPE serves as a last line of defense. Good laboratory techniques, procedures and appropriate containment equipment are the primary barriers against potential exposure to hazardous agents.

11.1 LABORATORY CLOTHING

11.1.1 Laboratory Clothing

- a. Laboratory clothing includes laboratory coats, scrub suits, and gowns.
- b. The lab clothing should be durable and provide protection of the skin from exposure to harmful agents.
- c. Long sleeved lab clothing should be used to minimize the contamination of skin or street clothes and to reduce shedding of micro-organisms, skin cells or hair from the arms. If proper precautions are not taken, contaminated clothes may carry infectious materials outside the laboratory and into other work areas, cafeterias, or to home.
- d. In procedures where splashes may occur, the lab clothing must be resistant to liquid penetration to protect street clothes from contamination.
- e. If the lab clothing is not disposable, it must be capable of withstanding sterilization, in the event it becomes contaminated.
- f. Change the lab clothing as soon as feasible whenever it is contaminated, soiled or torn. Upon overt exposure to agents at all levels of risk, immediately decontaminate the lab clothing and change into a clean set.
- g. Remove protective clothing and leave it in the laboratory before leaving for non-laboratory areas. Protective clothing worn within the laboratory should not be worn outside the facility to the library, cafeteria, toilets or other places accessible to the public.
- h. Do not take protective clothing home to launder. They should be discarded in the laboratory, disinfected or laundered by laundry services engaged by the department. All contaminated clothing should be decontaminated before being sent to the laundry or discarded. Treat contaminated areas with an appropriate disinfectant. Lab coats with extensive contamination may be placed in a biohazard bag and autoclaved.
- i. Provisions should be made for PPE to be provided to visitors and maintenance or security personnel, if applicable.

11.1.2 Shoes

- a. Shoes worn in the laboratory must be closed-toed and water resistant. Do not wear sandals, perforated, or cloth shoes.
- b. Depending upon the risk assessment, shoe covers or laboratory-specific shoes may be used:
 - when working with infectious agents the shoe covers can easily be removed, protecting and the laboratory specific shoes can be autoclaved; *or*
 - when working in tissue culture rooms shoe covers may be worn for the protection of cultures from contamination.
- c. In certain animal facilities personnel are required to wear overshoes or shoe covers to protect the animals in containment areas. Similarly, people who work with animals and do cage washing are required to wear protective water repellent shoes/boots.

- d. When handling heavy objects or equipment where there is a risk of the heavy objects falling or rolling onto the feet, such as in bottle-washing operations or animal care facilities, steel-toed safety shoes should be worn.

11.1.3 Gloves

- a. Gloves must be worn when working with biological agents and toxins (beginning with Risk Group 2 or equivalent risk) and physically hazardous agents. Breaks in the skin barrier of the hand (damaged cuticles, scrapes, micro-cuts, dermatitis, etc.) are common. Disposable (single use) gloves provide a barrier between infectious agents and the skin.
- b. Gloves should be comfortable and of sufficient length to prevent exposure of the wrist and/or forearm. When working with hazardous materials, the lower sleeve and the cuff of the laboratory garment should be overlapped by the glove. A long-sleeved glove or disposable arm-shield may be worn for further protection of the garment.
- c. The compatibility of the composition of the glove with the material to be handled should be taken into consideration when choosing a glove for a procedure (i.e. DMSO will penetrate latex gloves). See the [NUS Laboratory Chemical Safety Manual](#) (Appendix B, page 74) for more information about glove compatibility.
- d. Gloves may be fabricated of cloth, leather, natural and synthetic rubbers, or plastics depending on the hazards involved and the activities to be conducted. Consult the Safety Data Sheet of materials handled to select the appropriate type of glove.
- e. Check gloves for visible tears before use.
- f. Disposable gloves must not be washed or reused.
- g. Change gloves periodically and when soiled. Gloves must be disposed of when contaminated and removed when work with infectious materials is completed. Always wash hands after removing gloves.
- h. Gloves must never be worn outside the laboratory. Gloves shall be removed and hands washed before exiting the laboratory.
- i. Do not touch door handles, elevator buttons, telephones, computers or other clean surfaces or items with gloved hands.
- j. Normal disposable gloves will not prevent needle sticks or other percutaneous injuries.
- k. Surgical grade Kevlar gloves and stainless steel mesh gloves can provide protection against slices, scratches or cuts, but will not prevent direct puncture or needle stick injuries. Neoprene and other abrasive resistant gloves are cut resistant, but significantly reduce dexterity.
- l. Temperature-resistant gloves must be worn when handling hot material, dry ice or materials being removed from cryogenic storage devices.
- m. Chemical resistant gloves, such as nitrile or neoprene gloves must be worn when handling corrosive chemicals.
- n. In some instances double gloving may be appropriate e.g. work with highly infectious agents or for spill response.

11.2 FACE AND EYE PROTECTION

- a. Face protection is required for preventing splashes, sprays or splatters of infectious or other hazardous materials to the face.
- b. Face protection devices include safety goggles or safety glasses with solid side shields in combination with masks, chin length face shields or other splatter guards.
- c. Shields should cover the entire face, permit tilting back to clean the face if desired, and can be easily removed in the event of an accident.

- d. For laboratories where infectious agents or materials, corrosive and other injurious chemicals and materials are handled, eye protection shall be worn by all personnel (including all researchers, contractors, cleaners, and visitors) working in or visiting the laboratory.
- e. The nature of eye and/or face protection shall be determined through the risk assessment conducted by the Principal Investigator or Laboratory supervisor. Principal Investigator shall use the criteria mentioned in the [NUS Laboratory Chemical Safety Manual](#) (Section 6.4.2) for conducting the risk assessment and selection of eye and/or face protection.
- f. Contact lenses do not provide eye protection. It is recommended that contact lenses not be worn when working around biological agents, chemicals, fumes, and other hazardous material and dust particles since these items may become trapped in the space between the contact lens and the cornea. When contact lenses are worn, eye protection, such as tight fitting goggles, must be worn.

11.3 RESPIRATORY PROTECTION

- a. Infection via the respiratory system can occur by inhalation of respirable-sized aerosols of less than 5 μm .
- b. Respiratory protection can be worn to prevent exposure to potentially infectious aerosols. However, engineering controls, such as the use of biological safety cabinets, should always be considered as a first line of defense against respiratory infection when working with infectious organisms. Respiratory protection should only be considered as a second line of defense after feasible engineering controls have been put into place and additional controls are still needed.
- c. Acceptable methods of respiratory protection include: Properly fit-tested, HEPA filtered respirators (air purifying or powered air purifying) and N95 (or higher level) masks that are NIOSH-certified and FDA approved or the European equivalent, FFP2.
- d. Surgical masks provide no degree of respiratory protection as air may also be breathed in from the leaky sides. They are designed to protect the sterile field (and samples) from respiratory aerosols expelled by the wearer.
- e. Masks provide some protection only if they are used properly, are of the correct type for the situation or hazard, fit the persons using them and are properly maintained in good working condition.
- f. Personnel who require respiratory protection must enroll in the [NUS Respiratory Protection Programme](#) before using a respirator. Contact the Faculty Safety Officers for assistance in selection of equipment and proper usage.
- g. Accurate fit-testing is a key component of effective respirator use. The use of respirators requires medical clearance and fit-testing by the Occupational Health Clinic. Please submit the [NUS Biological Agents Work Medical Assessment Form](#) to Occupational Health Clinic, which is available on OSHE website.

CHAPTER 12 EMERGENCY RESPONSE DUE TO EXPOSURE TO POTENTIALLY INFECTIOUS MATERIALS

12.1 MANAGEMENT OF EXPOSURE TO POTENTIALLY INFECTIOUS MATERIALS

An "exposure incident" is defined as a specific eye, mouth, other mucous membrane, respiratory tract via inhalation, non-intact skin, or parenteral contact with blood or other potentially infectious materials that results from the performance of an employee's duties.

If a known or potential exposure incident has occurred, remove gloves and treat the affected area immediately. General actions to take following exposure incidents are as follows:

12.1.1 Percutaneous Injuries

- a. Percutaneous injuries include puncture wounds, needle stick injuries, cuts, abrasions, animal bites / scratches.
- b. For cuts and abrasions, wash area with soap and water for 1–2 minutes.
- c. For injuries with contaminated sharps and needle sticks, wash the affected area with antiseptic soap and warm water for 15 minutes. Apply an appropriate skin disinfectant.
- d. For animal bites and scratches, wash the affected area thoroughly with antiseptic soap and water for 15 minutes. For non-human primate injuries, a sponge scrub could be used to further clean the wound.
- e. Cover injured area with clean gauze.
- f. Seek medical attention as necessary for cuts and abrasions. **Seek prompt medical attention if injuries involve contaminated sharps and needle stick exposures to human material (blood, body fluids, tissues), as well as animal bites and scratches.**
- g. Report injuries to your PI or supervisor and complete accident / incident reports through [NUS Accident / Incident Reporting System \(AIRS\)](#) (Section 12.4). Report the cause of injuries and organisms involved (if any).
- h. Keep all medical records and accident/incident reports properly.

12.1.2 Splash to Face / Eye

- a. Flush affected area using an emergency eyewash for 15 minutes.
- b. Forcibly hold eyes open to ensure effective wash behind both eyelids.
- c. Seek prompt medical attention. Bring along relevant safety data sheets or other source of contaminant information to the physician's office.
- d. Report injuries to your PI or supervisor and complete accident / incident reports through [NUS Accident / Incident Reporting System \(AIRS\)](#) (Section 12.4).
- e. Keep all medical records and accident / incident reports properly.

12.1.3 Skin Contact

- a. Remove any contaminated clothing, jewelry, etc.
- b. Wash skin thoroughly with water e.g. by using a drench hose, emergency shower or faucet.
- c. Take care not to break the skin.
- d. Seek medical attention if necessary. Bring along relevant safety data sheets or other relevant information to the physician's office.

- e. Report injuries to your PI or supervisor and complete accident / incident reports through [NUS Accident / Incident Reporting System \(AIRS\)](#) (Section 12.4).
- f. Keep all medical records and accident / incident reports properly.

12.1.4 Ingestion of Potentially Infectious Material

- a. Seek medical attention. Provide information of material ingested and circumstances of the incident to the attending physician.
- b. Report injuries to your PI or supervisor and complete accident / incident reports through [NUS Accident / Incident Reporting System \(AIRS\)](#) (Section 12.4).
- c. Keep all medical records and accident / incident reports properly.

12.1.5 Aerosol Exposure

- a. Hold your breath and immediately vacate the area. Inform others.
- b. Remove Personal Protective Equipment (PPE) carefully. When removing the gown and gloves, make sure to turn the exposed areas inward. Wash hands well with soap and water.
- c. Post spill sign on lab entrances and allow aerosols to settle for at least 30 minutes.
- d. Inform the PI or supervisor immediately.
- e. Carry out appropriate decontamination procedure (see Section 12.3) after the appropriate time. The laboratory must be cleared from re-entry by the PI, biosafety officer or OSHE depending on the extent of decontamination.
- f. Seek medical attention. Provide information of material inhaled and circumstances of the incident to the attending physician.
- g. Report injuries to your PI or supervisor and complete accident / incident reports through [NUS Accident / Incident Reporting System \(AIRS\)](#) (Section 12.4).
- h. Keep all medical records and accident / incident reports properly.

12.2 MEDICAL ASSISTANCE

FOR EMERGENCY CARE DIAL 995 FOR AN AMBULANCE

For non-emergency cases, seek medical treatment at the University Health Centre (UHC)

In the event of exposure to biological materials or infectious agents resulting in possible infection, disease or illness, please contact the University Health Center (UHC) for a medical assessment during opening hours or proceed to the Accident & Emergency Units of Hospitals after office hours.

12.2.1 University Health Centre (UHC)

University Health Centre (UHC)

20 Lower Kent Ridge Road
University Health Centre, Level 1
Singapore 119080
Tel: (65) 6776 1631
E-mail: uhc_health@nus.edu.sg

Operating Hours

Mon – Thur	8.30 am – 6.00 pm
Fri	8.30 am – 5.30 pm
Sat, Sun & Public Holidays	Closed

Closed for lunch from 12.30 pm - 1.30 pm.

Last registration is 30 minutes before closing time.

For medical examinations that require lab tests and x-ray, please register between 8.30 am - 11.30 am or 1.30 pm - 4.30 pm.

12.2.2 National University Hospital (NUH)

In the event of critical injury or illness after office hours, proceed to the Accident & Emergency Unit of a nearby hospital. The nearest hospital in the vicinity of the University is:

National University Hospital (NUH)

Lower Kent Ridge Road

Singapore 119074

Main Line (24 hours general enquiries) Tel: (65) 6779 5555

Emergency Tel: (65) 6772 5000

12.2.3 Medical Emergencies

A medical emergency is an injury or illness that is acute and poses an immediate risk to a person's life or long term health. If an injury is a medical emergency, the lab personnel should be taken to the National University Hospital Accident and Emergency Department where initial assessment and emergency treatment will be provided.

- a. Call **995** for an ambulance in any life-threatening situation requiring immediate medical attention.
- b. Provide the following information:
 - Type of emergency and injuries;
 - Injured person's location, if applicable;
 - Your name, location and telephone number;
- c. Remain on line until the dispatcher disconnects the call.
- d. Check for hazards before entering location where emergency occurred.
- e. Initiate lifesaving measures if required and only if you are trained to do so.
- f. Do not move injured persons unless there is an immediate danger of further harm.
- g. Keep injured person warm.
- h. Remain with victim until medical assistance arrives.

12.2.4 Laboratory-Acquired Illness

If a laboratory personnel who works with or handles infectious material has acquired a laboratory acquired illness:

- a. Seek medical assistance **immediately**. Provide information on the infectious agent or material used in the laboratory.
- b. Report the illness to the Principal Investigator.
- c. Submit a report to OSHE via the online [NUS Accident / Incident Reporting System \(AIRS\)](#).
- d. Consult the medical care provider on fitness to return to work.

PIs should also assess the risk of exposure posed to fellow lab workers and other persons encountered by the affected personnel and determine whether their medical assessments are necessary.

12.2.5 Non-Emergency Medical Treatment

If non-emergency medical treatment is required following exposure,

- a. The medical treatment for the injury should be obtained as soon as possible following the injury.
- b. Bring along with you any relevant Safety Data Sheets or information of the contaminant that you were exposed to. If an incident report had been made earlier, you may present it to the attending doctor.

12.3 BIOLOGICAL SPILL RESPONSE

12.3.1 Spill Response Plan

In any spill scenario, the priority of actions should follow the order of People-Environment-Property. The highest priority is to provide aid to injured personnel and prevent access to spill area.

12.3.2 Biological Spill Kit

A biological spill kit is essential for labs working with infectious or potentially infectious agents classified at Risk Group 2 or higher and for groups working with large volumes (> 1 liter) of Risk Group 1 material.

A basic spill kit should include:

- a. Disinfectant appropriate for the infectious agent handled in the laboratory e.g. household bleach (see Section 8.2 Selecting Chemical Disinfectants)
- b. Bottle for making dilutions of disinfectant
- c. Forceps, autoclavable / disposable broom and dust pan, or tongs for handling sharps
- d. Sharps bin
- e. Paper towels or other suitable absorbent
- f. Biohazard autoclave bags for contaminated items
- g. Latex and/or nitrile gloves
- h. Face protection (eye wear and mask, or full face shield)
- i. Disposable lab-gown and shoe covers
- j. Spill response and clean-up procedure

Each spill kit should be tailored to meet the specific needs of each lab. It is the responsibility of the PI to ensure a well thought out spill kit is readily available and maintained.

One-time-use spill kits are also available from several safety supply sources. These kits contain everything needed for cleaning up and disposing of spills of biological agents and toxins.

Laboratories should have biological spill kits and trained laboratory staff that knows how to use them.

The spill kits should be strategically located close to the work areas so that they are easily accessible.

12.3.3 Spill Response Procedures

The degree of risk due to a spill depends on the volume of materials spilled, the concentration of organisms in the material spilled, the hazard of the organisms spilled, the route of infection of the organisms, the diseases caused by the organisms as well as the location of the spill.

12.3.3.1 Biological Spills in a BSL1 Laboratory

- a. Notify others in the area, to prevent contamination of additional personnel and environment
- b. Put on gloves and a lab coat.
- c. Cover spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its center.
- d. Leave in place for at least 30 minutes
- e. Wipe down any contaminated stationary, furniture or equipment with disinfectant.
- f. Pick up the towels and discard into a biohazard container. Use forceps, tongs or broom and dustpan to pick up any broken glass and place them into a sharps container. Refer to Section 7.3 of the manual for more information on the safe handling of sharps.
- g. Re-wipe the spill area with disinfectant.
- h. Remove gloves and thoroughly wash hands.
- i. Decontaminate reusable items or equipment.
- j. Inform laboratory personnel once the clean-up is over.
- k. Report the incident to OSHE online via the [NUS Accident / Incident Reporting System \(AIRS\)](#). Refer to Section 12.4 of the manual for more information.

12.3.3.2 Biological Spills in BSL2 Laboratory

- a. Hold your breath and leave the room immediately.
- b. Warn others to stay out of the spill area to prevent spread of contamination.
- c. Post a sign on the door warning others of the spill.
- d. Remove any contaminated clothing and put it into a biohazard bag for autoclaving.
- e. Wash hands and exposed skin and inform your PI or supervisor about the spill. Refer to Section 12.1 of the manual for more information on the medical management of biological fluids.
- f. Put on protective clothing (lab coat / disposable gowns, double gloves, N95 respirator, eye protection / goggles, shoe covers) and assemble clean-up materials.
- g. Wait 30 minutes before re-entering the contaminated area to allow dissipation / settling of aerosols.
- h. Cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its center. Refer to [Pathogen Safety Data Sheets and Risk Assessment](#) for appropriate type of disinfectant.
- i. Leave in place for at least 30 minutes. Refer to Safety Data Sheet for appropriate contact time.
- j. Collect all contaminated materials and discard in a biohazard container. Refer to Section 8.4 of this manual for more information on waste disposal.
- k. Use forceps, tongs, broom and dustpan to pick up any broken glass and place in a sharps container. Refer to Section 7.3 of the manual for more information on the safe handling of sharps.
- l. Re-wipe the spill area with disinfectant. Remove PPE in the correct order (i.e. safety glasses first and gloves last) and dispose as biohazard waste.
- m. Dispose biohazardous wastes according to procedures determined after adequate risk assessment. Refer to Section 8.4 of the manual for more information on waste disposal.
- n. Inform laboratory personnel once the clean-up is over.
- o. Report the incident to OSHE via the [NUS Accident / Incident Reporting System \(AIRS\)](#).
- p. Refer to Section 12.4 of the manual for more information

12.3.3.3 Spills inside a Biological Safety Cabinet (BSC)

- a. Leave the cabinet turned on.
- b. Inform others in the laboratory and notify the PI.

- c. Put on gloves and a lab coat.
- d. Spray or wipe cabinet walls, work surfaces, and equipment with disinfectant. If necessary, flood the work surface including drain pans and catch basins below the work surface with disinfectant.
- e. Wait at least 30 minutes.
- f. Soak up disinfectant and spill with paper towels.
- g. Drain catch basin into a container.
- h. Lift front exhaust grille and tray and wipe all surfaces.
- i. Ensure that no paper towels or solid debris are blown into the area beneath the grille.
- j. Please note that this procedure will not disinfect the air ducts, filters, fans or other interior parts of the BSC. If the entire interior of the BCS needs disinfection, contact the contracted certification vendor. Refer to Section 5.1 of this manual for more information on safe use of biological safety cabinets.
- k. Dispose all clean-up materials in the biohazardous waste container according to the procedure for biohazardous waste disposal.
- l. Wash hands with appropriate soap/disinfectant and any exposed surfaces thoroughly after the clean-up procedure.
- m. After the clean-up procedure, allow the cabinet to run for 10 minutes before resuming work.

12.3.3.4 Spills inside a Centrifuge

- a. Shut the affected centrifuge off and do not open for 30 minutes to allow the aerosols to settle.
- b. Warn others in the laboratory and notify the PI.
- c. Wear the appropriate PPE before opening the centrifuge lid.
- d. Use a squeeze bottle to apply disinfectant to all contaminated surfaces within the chamber taking care to minimize splashing.
- e. Allow 30 minutes contact time before cleaning up the chamber.
- f. Remove the buckets and rotors to a biosafety cabinet and disinfect and clean them according to the manufacturer's instructions. Refer to Section 5.6 of the manual for more information on centrifuge safety.

12.3.3.5 Spills during Transport of Biohazardous Material

- a. All biohazardous material must be packed in a well-sealed primary container and a leak-proof secondary container containing absorbent material before it leaves the laboratory to be transported to another location. Refer to Chapter 9 of this manual for more information on transfer and transport of regulated and non-regulated biological materials.
- b. The exterior of the secondary container must be wiped down with appropriate disinfectant so that it can be transported without wearing gloves.
- c. Carry a spill kit if possible, or at least some paper towels and an appropriate disinfectant while transporting biohazardous material.
- d. In case of a spill, notify people in the vicinity and clean-up the spill according to spill response procedure.

12.3.4 Waste Disposal

Materials used in biological spill clean-up must be disposed of as biohazardous waste. This may or may not be autoclaved before it is collected by the licensed waste collector depending on the risk level of the biological agent and the institution or departmental policy. Refer to Section 8.4 of this manual for more information regarding waste disposal.

12.4 ACCIDENTS AND INCIDENTS REPORTING

Laboratory events that might create hazards, exposures, or accidents requiring reporting include:

- Accidents during work with biological agents and toxins that result in physical injury, cuts, burns, abrasions, or fractures. The injured site could be contaminated with the biohazardous agent in use.
- Incidents occurring during the handling of biohazardous agents, infected specimens, or animals that could allow the undesired transfer of the agent to the lab personnel or release of the agent to the environment e.g. biological spills, exposure to aerosols and penetration of agents through the skin.

All accidents, known exposures, and potential hazards should be identified and reported in order to control the biohazards and contain the organisms involved as well as implement preventive and corrective measures to prevent such accidents from happening.

All incidents or accidents have to be notified to OSHE via the online [NUS Accident / Incident Reporting System \(AIRS\)](#).

The AIRS report can be submitted by either the injured staff/student, staff in-charge of visitor or contractor, or his or her supervisor/representative if the staff or student is unfit/unable to do the initial report.

OSHE and/or the Faculty Safety and Health Officer, in cooperation with the PI and his/her staff, will conduct the necessary investigation of any laboratory accident. The goal of the investigation is to prevent similar accidents as well as to assess the circumstances and number of personnel who may have been exposed to the agent in question.

12.5 EMERGENCY RESPONSE PLANS

Departments are encouraged to develop an emergency response plan which covers contingencies which may arise in the event of an accidental exposure.

The fundamental rule in dealing with a biological spill is to be prepared. Establish an emergency spill response plan. It should consist of a step-by-step procedure to follow if a spill occurs. Spill kit materials should be present in proximity to the area where biohazardous materials are handled. Identify the biohazard risks involved on the site and the types of potential spills or emergencies which can occur.

The emergency response plan should include the following information:

- a. Location of first aid kits, holding area to house the injured persons and first-aiders available;
- b. Location of spill kits – biological and chemical;
- c. Location of emergency showers and eye-washes;
- d. Location of fire extinguishers and the fire-fighting team;
- e. The type of ventilation system serving the lab, corridors and the building;
- f. Location of where the exhaust air is discharged from the fume hoods and biological safety cabinet exhaust;
- g. Location of where the biohazard work areas and storage areas of biohazardous materials;

- h. Evacuation routes and procedures to be used in the event of an emergency with biohazardous materials;
- i. Evacuation routes of the injured person to a holding area and to the pickup point of the ambulance;
- j. Established procedures for safe handling, storage and disposal of biohazardous materials to minimize accidental release and to avoid conditions which might lead to accidental spills;
- k. Procedures for dealing with exposure to biohazardous materials;
- l. Any agent-specific post exposure treatment protocol; *and*
- m. Procedures for accident / incident reporting.

CHAPTER 13 BIOSECURITY

The objective of biosecurity is to prevent loss, theft or misuse of micro-organisms, biological materials, and research-related information. This is accomplished through institutional and personal measures by limiting access to facilities, research materials and information.

13.1 BIOSECURITY PROGRAMME

13.1.1 Risk Management Methodology

A risk management methodology can be used to identify the need for a biosecurity programme and ensures that the protective measures provided, and the costs associated with that protection, are proportional to the risk. Through risk assessments, information is gathered regarding the type of organisms available, their physical location, the personnel who require access to them, and the identification of those responsible for them. This information can be used to assess whether an entity possesses biological materials that are attractive to those who may wish to use them improperly.

13.1.2 Administrative Controls

A specific laboratory biosecurity programme must be prepared and implemented for each facility according to the requirements of the facility and the type of laboratory work conducted. Consequently, laboratory biosecurity activities should be representative of the institution's various needs and should include input from scientific directors, principal investigators, biosafety officers, laboratory scientific staff, maintenance staff, administrators, information technology staff, and law enforcement agencies and security staff if appropriate.

Laboratory biosecurity measures should be based on a comprehensive programme of:

- a. accountability for pathogens and toxins that includes an updated inventory with storage location,
- b. identification of personnel with access,
- c. description of use,
- d. documentation of internal and external transfers within and between facilities,
- e. any inactivation and/or disposal of the materials,
- f. identifying, reporting, investigating and remediating breaches in laboratory biosecurity, including discrepancies in inventory results.

The involvement and roles and responsibilities of public health and security authorities in the event of a security infraction must be clearly defined.

In some cases, biosecurity practices may conflict with biosafety practices, requiring personnel and management to devise policies that accommodate both sets of objectives. For example, signage may present a conflict between the two programmes. Standard biosafety practice requires that signage be posted on laboratory doors to alert people to the hazards that may be present within the laboratory. The biohazard sign normally includes the name of the agent, specific hazards associated with the use or handling of the agent and contact information for the investigator. These practices may conflict with security objectives. Therefore, biosafety and biosecurity considerations must be balanced and proportional to the identified risks when developing institutional policies.

13.1.3 Training & Emergency Response

Biosecurity training should incorporate training programmes that inform and educate individuals regarding their responsibilities within the laboratory and the institution. Practice drills should address a variety of scenarios such as loss or theft of materials, emergency response to accidents and injuries, incident reporting and identification of and response to security breaches. These scenarios may be incorporated into existing emergency response drills such as fire drills or building evacuation drills associated with bomb threats. Incorporating biosecurity measures into existing procedures and response plans often provides efficient use of resources, saves time and can minimize confusion during emergencies.

In summary, security procedures should become a routine part of laboratory work, just as aseptic techniques and other safe microbiological practices have been incorporated into daily practices in the laboratory. Assessment of the suitability of personnel, security-specific training and rigorous adherence to pathogen protection procedures are reasonable means of enhancing laboratory biosecurity. All such efforts must be established and maintained through regular risk and threat assessments, and regular review and updating of procedures. Checks for compliance with these procedures, with clear instructions on roles, responsibilities and remedial actions, should be integral to laboratory biosecurity programmes and national standards for laboratory biosecurity.

13.2 PROTECTED AREAS AND PROTECTED PLACES ACT (PAPP)

Any Principal Investigator who intends to possess First Schedule Part II, Second Schedule & Fifth Schedule biological agents and toxins, must ensure that the facility is gazetted as a protected place under the [Protected Areas and Protected Places Act \(PAPP\)](#), due to their bioterrorism potential. These agents require a special facility inside a protected area which must be approved by Ministry of Home Affairs.

A “protected area” is defined any area declared to be a protected area and “protected place” means any premises declared to be a protected place by virtue of the Protected Areas and Protected Places Act.

In addition, the possession and use of First Schedule and Second Schedule biological agents also require a certified BSL3 facility, which has to be certified by MOH Approved Facility Certifiers (AFCs).

Please refer to the “[Application for Gazetting as a Protected Place under the Protected Areas and Protected Places Act for the Possession of Biological Agents and Toxins listed in the BATA](#)” on guidelines for the application for Protected Areas and Protected Places.

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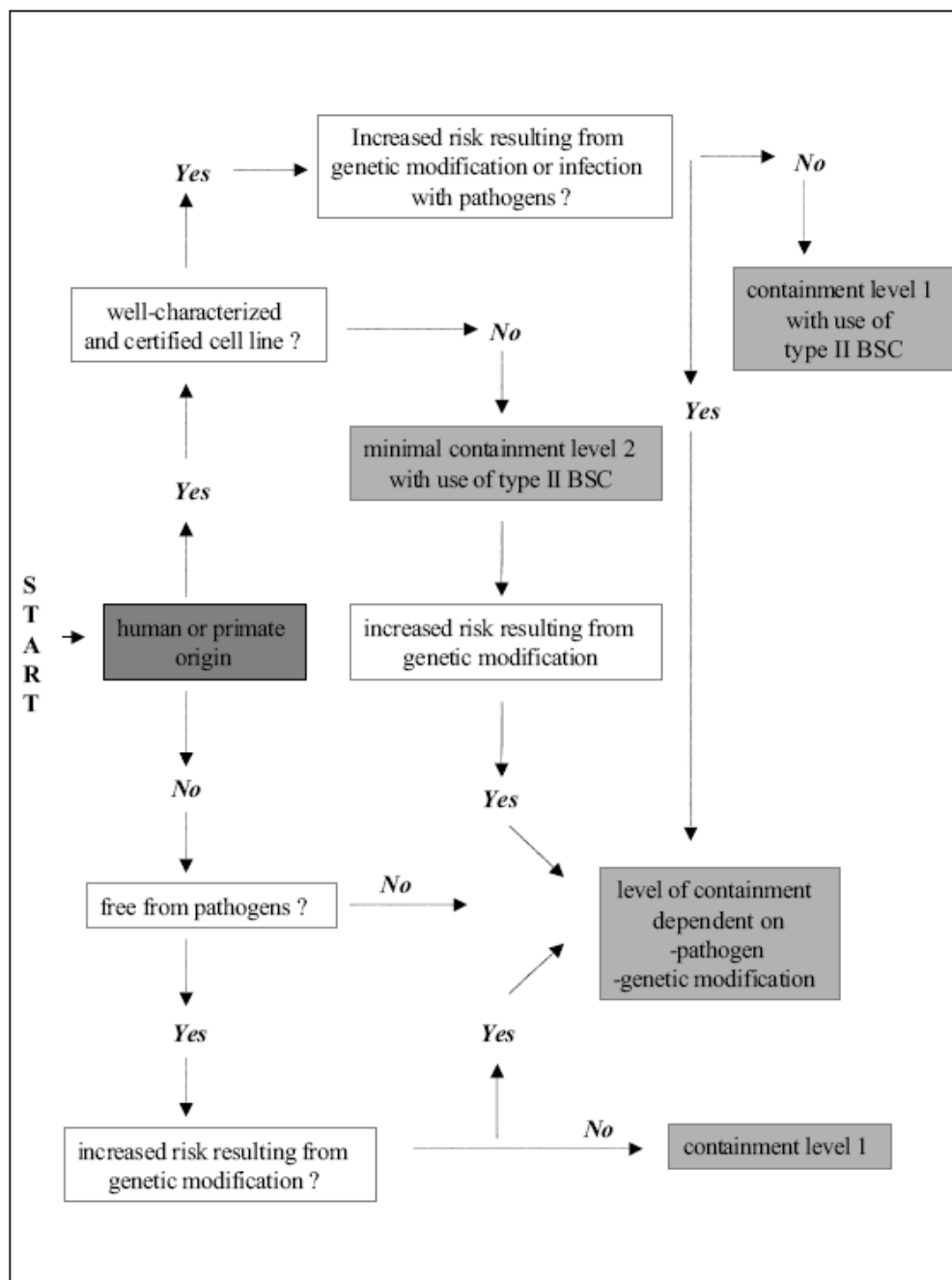
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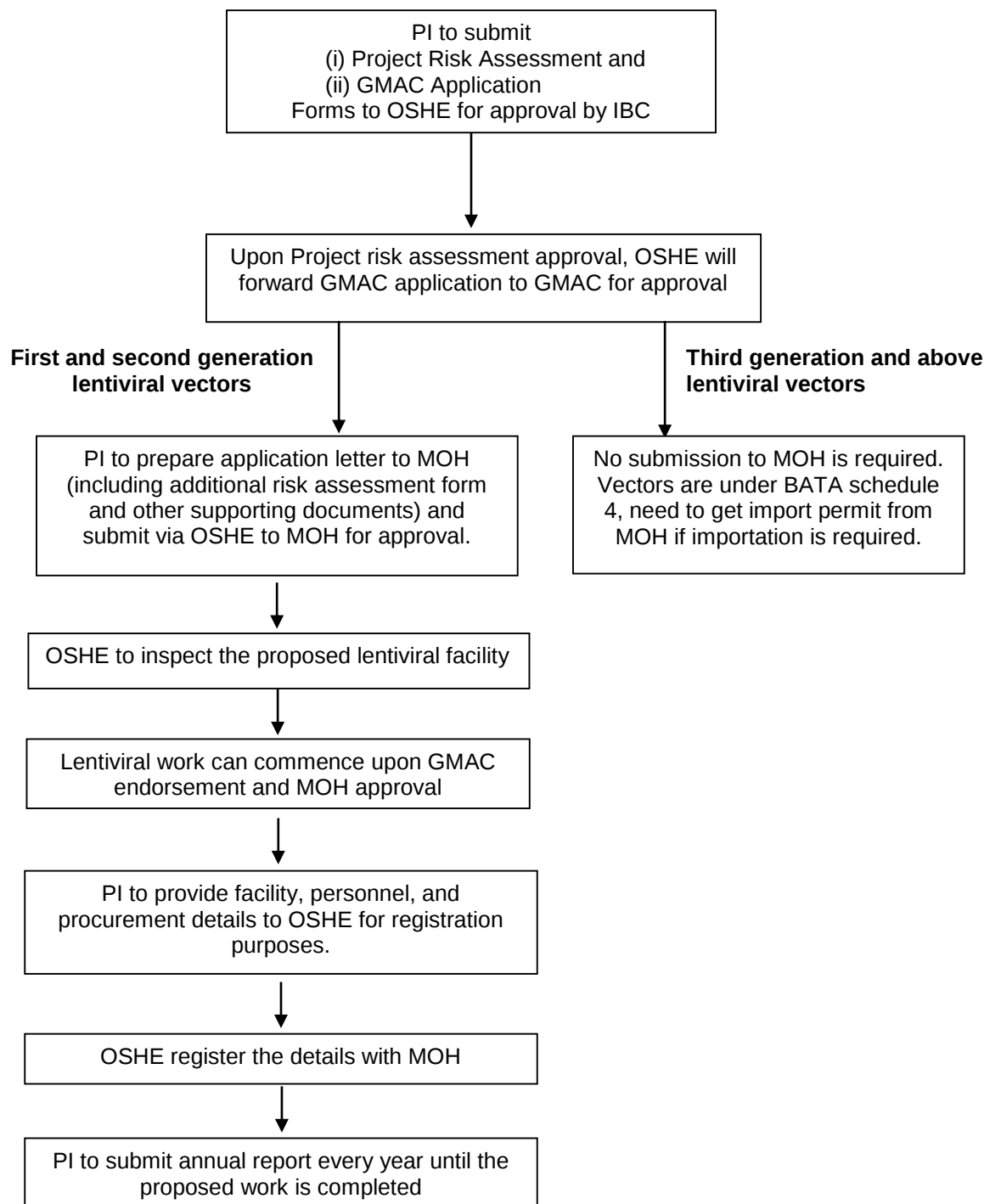
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APPENDICES

Appendix A.1 Guidelines for selection of containment level for cell lines (adapted from *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition).



Appendix A.2 Flowchart for obtaining approval for work involving HIV based lentiviral vectors



Appendix A.3 Template for application letter to MOH Regulatory Policy Branch (Biosafety Team) to seek approval for possession and use of non-advanced generation lentiviral vectors

(Date)

Dr Se Thoe Su Yun
Head
Regulatory Policy Branch (Biosafety Team)
Ministry of Health
College of Medicine Building
16 College Road
Singapore 169854

Dear Dr Se Thoe,

Application to seek approval for possession and permission to work with (XXX) at the National University of Singapore

[Paragraph 1: Introduction - why need approval]

In accordance with the Biological Agents and Toxin Act, we wish to seek approval for the possession and use of *(agent or its components/ derivatives)* in *(location of work- Dept., National University of Singapore)*

[Paragraph 2: Principal investigator details]

(Provide background of PI, experience with working on this agent, experience and training of others working on this agent in PI's group)

[Paragraph 3: Details of research work]

(Describe current research project, source of grant, rationale, description of research work to be carried out with agent)

[Paragraph 4: References]

(Cite references if any on similar work, by PI or others)

[Paragraph 5: Agent details]

(Risk group classification of agent e.g. under ABSA / other organizations, cite references for containment of agent etc.)

[Paragraph 6: Risk Assessment]

(List (i) hazards and risks of agent and procedures involved (ii) mitigating measures and other precautions taken (containment facility / equipment, safe work procedures, training, medical surveillance)

[Paragraph 7: Appendix]

(Attach SOPs and other supporting documents if any like training certificates.)

[Closing note]

As required by the act, we thus seek the MOH Regulatory Policy Branch (Biosafety Team)'s approval for the possession of (XXX) and to work with this biological agent in our laboratory at NUS. To aid in the approval process, we enclose the following documents for your review:

- NUS Project Risk Assessment approved by the NUS Institutional Biosafety Committee
- additional risk assessment form for non-advanced generation lentiviral work
- proposal form submitted to the Genetic Modification Advisory Committee (GMAC)
- Lab SOP for working with non-advanced generation lentiviral vectors
- *give details if any other supporting documents are attached*

Yours sincerely,

(PI's name)
(Department)
(Contact Details)

Cc:

(Prof / Assoc Prof XXX)
Head/ Director, Department of (XXX), Faculty of (XXX)

Prof / Assoc Prof XXXX
Chairman, Institutional Biosafety Committee

Dr Peck Thian Guan
Director, OSHE

Appendix A.4 Additional Risk Assessment Form

PROJECT RISK ASSESSMENT [for Non-Advanced Generation Lentiviral vector (LV)]

Provide detail information, and supporting documents, where applicable, on the following:

1. Describe how access to the laboratory where LV work is conducted is controlled (e.g. card access, accessible from uncontrolled area or another controlled area, etc.).

2. Location of the following equipment:

- Autoclave

(If the autoclave is located outside LV room, describe how waste is transported there, how access is being controlled where waste is stored there before autoclaving)

- Freezer ☐ locked ☐ unlocked

(If the freezer is located outside LV room, describe the access control of that location and the transportation procedure)

- Incubator ☐ locked ☐ unlocked

(Please note that one incubator is assigned to only LV work. If the incubator is located outside LV room, describe the access control of the incubator)

3. Replication Competence of Lentivirus (RCL) Test

- Who will perform the test? Provide test method and test report.

What cells will be used to do the RCL test?

NOTE: Once LV work has started, Replication Competence Test has to be performed every 6 months, with the reports submitted to OSHE at year end.

4. Provide a list of the plasmids in the packaging-mix system and information e.g. the map on those plasmids

Appendix A.5 Table for submitting details of personnel proposed to be involved in second generation lentiviral work

Work Site (Block and unit number):

Proposed start date of project:

Details of personnel working in the lab facility

NAME OF LAB PERSONNEL	NRIC/FIN	NATIONALITY	CONTACT NO.	DESIGNATION	LENGTH OF EMPLOYMENT	AREA OF WORK

Appendix A.6 Biological Safety Risks when working with Lentivirus / Lentiviral Vector

- Lentiviral particles are usually pseudotyped with the vesicular stomatitis virus glycoprotein (VSVG), an envelope protein which gives the virus the ability to infect many human and mammalian cell types. The skin affords some protection, but the virus may enter the body through injured skin or wounds or by infecting mucosal surfaces (eyes, nose, mouth, etc.). Pseudotyping with other proteins will change the tropism of the viral particles and therefore alter the risk.
- A lentivirus is an enveloped virus, so it is susceptible to inactivation by sufficiently long treatment with 70% ethanol, freshly diluted 10% bleach, or other efficacious disinfectants.
- The direct effect of the lentiviral vector on the infected cell will depend in part on the protein that the virus is engineered to produce (the product of the transgene).
- Lentiviruses can integrate into the chromatin of non-dividing cells.
- Lentiviral vectors are engineered to be replication incompetent so that the infection will not spread beyond the site of injection. However there is a risk of generating infective replication competent virus through recombination. Advanced generation lentiviral vectors, (such as those belonging to third and higher generations, in which 4 plasmids are used to produce the viral particles or where a deletion is created in the long terminal repeats (LTR)), upon integration have a lower risk of generating replication competent virus than non-advanced generation lentiviral vectors. Therefore the substitution of advanced generation lentiviral vectors is encouraged where practical.
- Apart from the generation of the vector, transgene should also be considered in assessing the risks and coming up with controls. Therefore, if the transgene encodes a biological toxin, an oncogene, a cells cycle regulator or an inhibitor of a tumor suppressor (e.g. siRNA for a tumor suppressor) more control measures may be needed.
- Lentivirus cannot replicate in rodents, but as rodents can shed virus for days after infection, the infection of rodents must be conducted under BSL-2 containment. If the animals have been transplanted with human cells, and if replication competent virus is generated, the virus could replicate in the transplanted cells.
- As lentiviral vectors are derived from HIV which is a Bloodborne Pathogen (BBP), the use of sharp instruments should be restricted or eliminated. In the circumstances that sharps, such as needles cannot be avoided, (such as the direct inoculation of lentiviral vectors into the animals) necessary precautions must be taken during the procedures and medical assistance shall be sought for accidental exposure. The potential consequence of **integration can lead to insertional mutagenesis or transactivation of the neighboring genes.**

Appendix B.1 Classes of Organisms Ranked in Order of Susceptibility to Disinfectants

Least Susceptible  Most Susceptible	Prions
	Bacteria with Spores (<i>B. subtilis</i> , <i>C. tetani</i> , <i>C. difficile</i> , <i>C. botulinum</i>)
	Protozoa with Cysts (<i>Giardia lablia</i> , <i>Cryptosporodium parvum</i>)
	Mycobacteria (<i>M. tuberculosis</i> , <i>M. avium-intracellulare</i> , <i>M. chelonae</i>)
	Non-enveloped Viruses (Coxsackievirus, poliovirus, rhinovirus, Norwalk-like virus, Hepatitis A virus)
	Fungi (<i>Candida sp.</i> , <i>Cryptococcus sp.</i> , <i>Aspergillus sp.</i> , dematophytes)
	Vegetative Bacteria (<i>Staphylococcus aureus</i> , <i>Salmonella typhi</i> , <i>Pseudomonas aeruginosa</i> , coliforms) Enveloped Viruses (<i>Herpes simplex</i> , varicella-zoster virus, cytomegalovirus, measles virus, mumps virus, rubella virus, influenza virus, respiratory syncytial virus, Hepatitis B & C viruses, hantavirus and HIV)

Appendix B.2 Disinfection Selection Table

Disinfectant Selection Table								
<i>Compound</i>	<i>Chlorine</i> 0.01-5%	<i>Iodine</i> Iodophor 0.5-5%	<i>Chlorhexidine</i> 0.05-0.5%	<i>Alcohol</i> 70-95%	<i>Oxidizing</i> 0.2-3%	<i>Phenol</i> 0.2-3%	<i>Quaternary</i> <i>Ammonium</i> 0.1-2%	<i>Aldehyde</i> 1-2%
Examples	Clorox	Tincture / Providone	Novalsan		VikronS	Lysol	Roccal-D	Wavicide
Bactericidal	Good	Good	Very Good	Good	Good	Good	Good	Very Good
Virucidal	Very Good	Good	Very Good	Good	Good	Fair	Fair	Very Good
Envelope Viruses	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Non-Envelope Viruses	Yes	Yes	No	No	Yes	No	No	Yes
Bacterial Spores	Fair	Fair	Poor	Fair	Fair-Good	Poor	Poor	Good
Fungicidal	Good	Good	Fair to Good	Fair	Fair	Good	Fair	Good
Protozoal Parasites	Fair strong Conc	Poor	Poor	Poor	Poor	Poor	Fair (Ammonia)	Good
Effective in Organic Matter	Poor	Fair	Fair	Fair	Poor	Good	Poor	Good
Inactivated by soap	No	No and Yes	No	No	No	No	Yes	No
Effective in Hard water	Yes	No	Yes	Yes	Yes	Yes	No	Yes
Contact Time (minutes)	5-30	10-30	5-10	10-30	10-30	10-30	10-30	10-600
Residual activity	Poor	Poor	Good	Fair	Poor	Poor	Fair	Fair

Adapted from Kennedy J, Bek J, Griffin D. (2005). *Selection and Use of Disinfectants*. University of Nebraska-Lincoln Extension.

Appendix B.3 Antimicrobial Agent and Neutralizing and/or Inactivating Agent

<i>Antimicrobial Agent</i>	<i>Neutralizing and/or inactivating agent^b</i>
Alcohols	None (dilution)
Alcohol-based hand gels	Tween 80, saponin, histidine and lecithin
Amoxycillin	β -Lactamase from <i>Bacillus cereus</i> ^c
Antibiotics (most)	None (dilution, membrane filtration ^d , resin adsorption ^e)
Benzoic Acid	Dilution or Tween 80 ^f
Benzyl penicillin	β -Lactamase from <i>Bacillus cereus</i> ^c
Bronopol	Cysteine hydrochloride
Chlorhexidine	Lubrol W and egg lecithin or Tween 80 and lecithin (Letheen)
Formaldehyde	Ammonium ions
Glutaraldehyde	Glycine
Halogens	Sodium thiosulphate
Hexachlorophene	Tween 80
Mercurials	Thioglycollic acid (-SH compounds)
Phenolics	Dilution or Tween 80
QACs	Lubrol W and lecithin or Tween 80 and lecithin (Letheen)
Sulphonamides	p-Aminobenzoic acid

^a Other than dilution – adapted from Hugo & Russell (1998)

^b D/E neutralizing media – adequate for QACs, phenols, iodine and chlorine compounds, mercurials, formaldehyde and glutaraldehyde

^c Other appropriate enzymes can be considered – e.g. inactivating or modifying enzymes for chloramphenicol and aminoglycosides, respectively.

^d Filter microorganisms onto membrane, wash, transfer membrane to growth medium.

^e Resins for the absorption of antibiotics from fluids are available.

^f Tween 80 (polysorbate 80)

Adapted from Hugo WB, Russell AD. (1998). Evaluation of non-antibiotic antimicrobial agents. In: Pharmaceutical Microbiology (eds WB Hugo, AD Russell). 6th edition, pp. 229-255. Blackwell Science, Oxford

Appendix B.4 Classification of Biohazardous Wastes Based on Treatment or Disposal Methods

	Classification	Examples
1	Sharps	Blood-drawing equipment, needles, syringes, slides, Pasteur pipettes, capillary tubes, broken glass and scalpel blades.
2	Autoclavable wastes	All laboratory specimens or materials consisting of, containing, or contaminated with blood, plasma, serum, urine, faeces or other human or animal tissues or fluids, as well as inoculated media, cultures, contaminated paper wastes such as wrappers and towels and other potentially infectious materials
3	Wastes for incineration or cremation	Animal carcasses, human tissues, organs, etc.
4	Chemical decontaminated wastes	Wastes that cannot be autoclaved.
5	Environmentally benign and not contaminated with infectious agents for disposal into sewer	Uninoculated liquid medium, nutrient fluids, tissue cultures that do not contain any infectious agents.

Appendix C.1 Definitions as defined in IATA Dangerous Goods Regulations, and United Nations Recommendations on the Transport of Dangerous Goods - Model Regulations

Class 6, Division 6.1 Toxic Substances

Toxic Substances are substances liable either to cause death or serious injury or to harm human health swallowed or inhaled or by skin contact.

Toxins from plant, animal or bacterial sources which contain infectious substances, or toxins that are contained in infectious substances, shall be classified in Division 6.2.

Class 6, Division 6.2 Infectious Substances

Infectious substances are substances which are known or are reasonably expected to contain pathogens. Pathogens are defined as micro-organisms (including bacteria, viruses, rickettsiae, parasites, fungi) and other agents such as prions, which can cause disease in humans or animals.

Biological products are those products derived from living organisms which are manufactured and distributed in accordance with the requirements of appropriate national authorities, which may have special licensing requirements, and are used either for prevention, treatment, or diagnosis of disease in humans or animals, or for development, experimental or investigational purposes related thereto. They include, but are not limited to, finished or unfinished products such as vaccines.

Cultures are the result of a process by which pathogens are intentionally propagated. This definition does not include patient specimens as defined below.

Patient specimens are those collected directly from humans or animals, including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluid swabs, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention.

Medical or clinical wastes are wastes derived from the medical treatment of animals or humans or from bio-research.

Class 9 Miscellaneous Dangerous Substances and Articles, including environmentally hazardous substances

Genetically modified microorganisms (GMMOs) and organisms (GMOs) are microorganisms and organisms in which genetic material has been purposely altered through genetic engineering in a way that does not occur naturally.

Genetically modified microorganisms not meeting the definition of infectious substance are classified in Class 9 (Miscellaneous dangerous substances and articles, including environmentally hazardous substances). GMMOs and GMOs are not subject to dangerous goods regulations when authorized for use by the competent authorities of the countries of origin, transit and destination. Genetically modified live animals shall be transported under terms and conditions of the competent authorities of the countries of origin and destination.

Appendix C.2 Biological Hazard Sign for Biological Agents or Toxins as specified in Biological Agents and Toxins Act for Transport within Singapore

Biological Hazard for Biological Agents (Transport within Singapore)



Notes:

- | | |
|---|--|
| 1. Symbol | : 3 crescents superimposed on a circle |
| 2. Inscription | : 6 |
| 3. Symbol and inscription | : black |
| 4. Background | : white |
| 5. Dimensions (applicable only for signs used on transporting conveyance) | |
| (a) vehicle exceeding 3.5 metric tons | : 25 cm x 25 cm |
| (b) vehicle not exceeding 3.5 metric tons | : 11 cm x 11 cm |

Biological Hazard Sign for Toxins (Transport within Singapore)



Notes:

- | | |
|---|------------------------|
| 1. Symbol | : skull and crossbones |
| 2. Inscription | : 6 |
| 3. Symbol and inscription | : black |
| 4. Background | : white |
| 5. Dimensions (applicable only for signs used on transporting conveyance) | |
| (a) vehicle exceeding 3.5 metric tons | : 25 cm x 25 cm |
| (b) vehicle not exceeding 3.5 metric tons | : 11 cm x 11 cm |

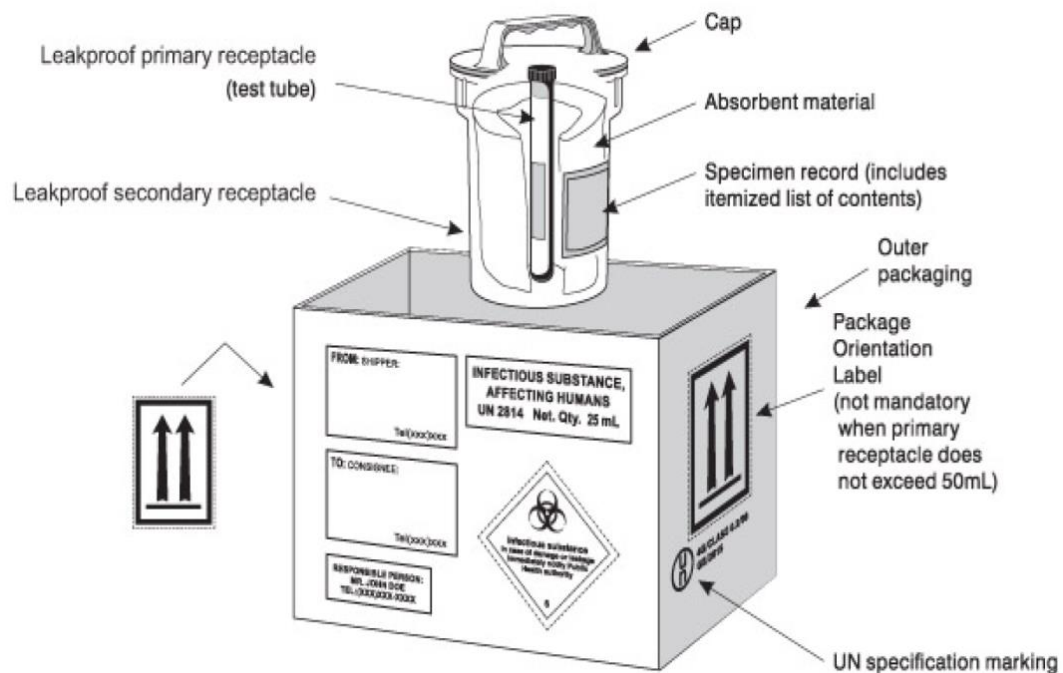
Appendix C.3 Classifications of Dangerous Goods

Dangerous goods are classified into 9 classes as follows irrespective of carrier:

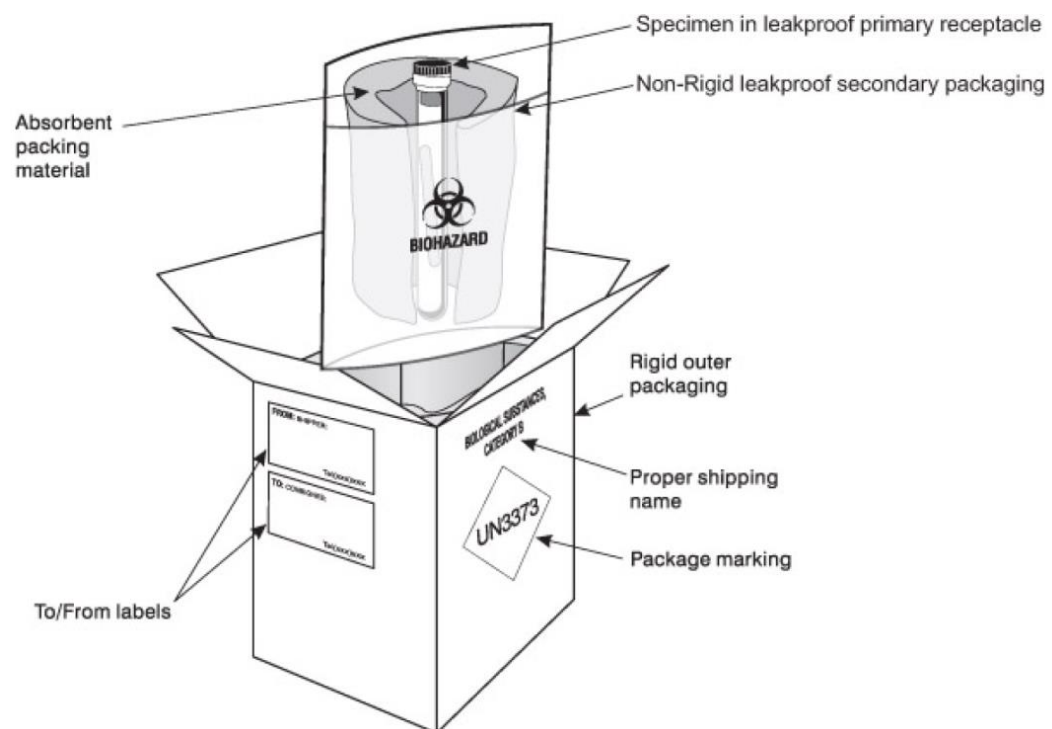
Class		Division	
1	Explosives	1.1	Explosives with a mass explosion hazard
		1.2	Explosives with a blast/projection hazard
		1.3	Explosives with a minor blast hazard
		1.4	Explosives with a major fire hazard
		1.5	Blasting agents
		1.6	Extremely insensitive explosives
2	Gases	2.1	Flammable Gases
		2.2	Non-Flammable, Non-Toxic Gases
		2.3	Toxic Gases
3	Flammable Liquids	-	-
4	Flammable Solids	4.1	Flammable Solids
		4.2	Substances liable to spontaneous combustion
		4.3	Substances which in contact with water emit flammable gases
5	Oxidizing Substances and Organic Peroxides	5.1	Oxidizing Substances
		5.2	Organic Peroxides
6	Toxic and Infectious Substances	6.1	Toxic Substances
		6.2	Infectious Substances
7	Radioactive Materials	-	-
8	Corrosive Substances	-	-
9	Miscellaneous Dangerous Substances and Articles, including environmentally hazardous substances	-	-

Appendix C.4 Examples of packing and marking (International Air Transport Association)

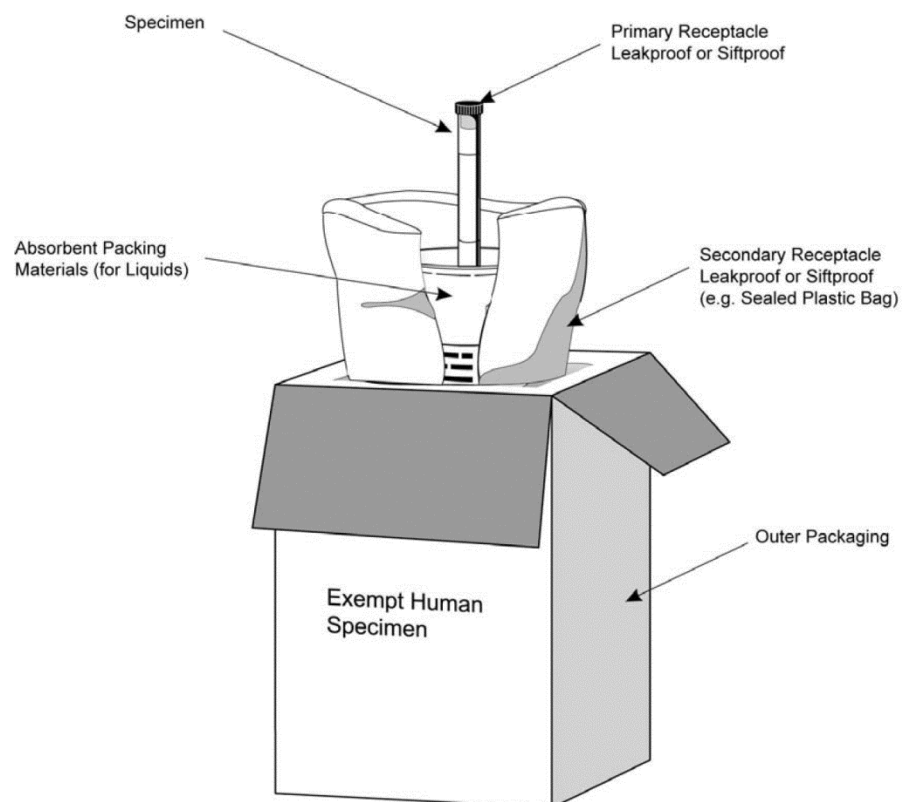
(source: IATA Guidance Document for Transport of Infectious Substances)



Example of packing and marking for Category A Infectious Substances

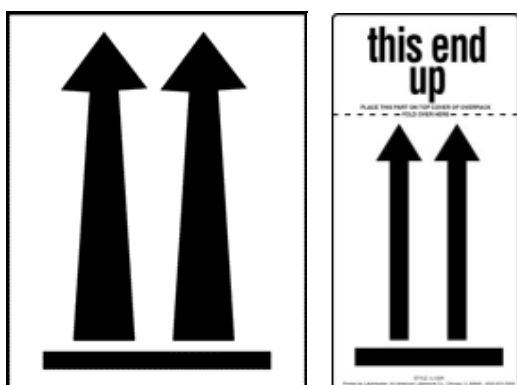


Example of packing and marking for Category B Infectious Substances



Example of packing and marking for Exempt Specimen

Appendix C.5 Examples of Labels and Marking (International Air Transport Association)



Examples of orientation label, “This End Up”



Hazard label for Toxins (Class 6)



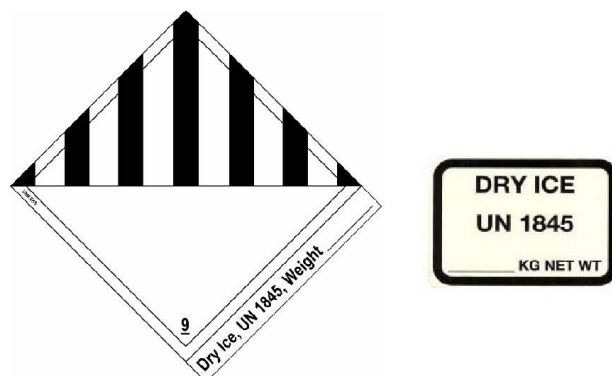
Hazard label for Category A Infectious Substances (Class 6), and for genetically modified microorganisms and organisms that meet the definition of an infectious substance, Category A



Diamond-shaped mark for “Biological Substance, Category B” and for genetically modified microorganisms and organisms that meet the definition of an infectious substance, Category B



Hazard label (Class 9 Miscellaneous Dangerous Goods) for certain noninfectious genetically modified microorganisms and organisms (UN 3245) and for dry ice (UN 1845)



Example of hazard label for dry ice (Class 9 Miscellaneous Dangerous Goods), and a label indicating the quantity of dry ice

Appendix C.6 Summary of shipping information for air transport of biological materials

(source: *Guidance on Regulations for the Transport of Infectious Substances 2011-2012* by World Health Organization)

Proper Shipping Name	UN Number	Hazard Class	Packing Group	Packing Instructions	Max. Net qty./pkg for Passenger Aircraft	Max. Net qty./pkg for Cargo Aircraft
Toxins, extracted from living sources, liquid, n.o.s.	UN 3172	6.1	I	652	1 L	-
			II	654	5 L	-
			III	655	60 L	-
			I	658	-	30 L
			II	662	-	60 L
			III	663	-	220 L
Toxins, extracted from living sources, solid, n.o.s.	UN 3462	6.1	I	666	5 kg	-
			II	669	25 kg	-
			III	670	100 kg	-
			I	673	-	50 kg
			II	676	-	100 kg
			III	677	-	200 kg
Infectious substance, affecting humans	UN 2814	6.2	-	620	50 ml or 50 g	4 L or 4 kg
Infectious substance, affecting animals <i>only</i>	UN 2900	6.2	-	620	50 ml or 50 g	4 L or 4 kg
Biological substance, Category B	UN 3373	6.2	-	650	1 L (primary receptacle); 4 L or 4 kg (outer packaging)	1 L (primary receptacle); 4 L or 4 kg (outer packaging)
Genetically modified microorganisms <i>or</i> Genetically modified organisms*	UN 3245	9	-	959	100 ml or 100 g (inner packaging); No limit	100 ml or 100 g (inner packaging); No limit
Carbon dioxide, solid (Dry Ice)	UN 1845	9	III	954	200 kg	200 kg

n.o.s. = not otherwise specified

UN = United Nations

*non-infectious; that do not meet the definition of toxic substances/infectious substances

Appendix C.7 Shipper's Declaration for Dangerous Goods

(source: IATA at <http://www.iata.org/whatwedo/cargo/dgr/Pages/download.aspx>)

- a. [Shipper's Declaration for Dangerous Goods](#)
- b. Checklist for Non-Radioactive Shipment
- c. [Checklist for Dry Ice](#)

Guide to Shipper's Declaration for Dangerous Goods:

The person signing these papers is assuming full responsibility for the package. The shipper must fill out the form. Include the following information:

Shipper - full name, address, telephone and contact person

Consignee - full name, address, telephone and contact person

Air Waybill Number - The carrier will have this number for you

Page of - enter as appropriate

Aircraft Limitation - delete the box that does not apply to this shipment. Volume limits for shipments that travel on passenger aircraft rarely apply to research packages.

Airport of Departure - The shipping company will supply this information

Airport of Destination - The shipping company will supply this information

Shipment Type - delete the box that does not apply

Nature and quantity of Dangerous Goods - Complete this section with the proper shipping name (Infectious substance, affecting humans [Ebola virus]). If using dry ice, add Dry Ice.

Class or Division - this comes from a chart published by both DOT and the International Air Transport Association. For Infectious substances, the number is 6.2. To ship other dangerous goods, contact the carrier or faculty safety officer for assistance.

UN or ID number - as on the package markings section. Always include the prefix "UN"

Packing group - does not apply to infectious substances (the packing group for dry ice is III).

Subsidiary Risk - does not apply to infectious substances.

Quantity and type of packing - the information on the complete packing list, enter the total net quantity.

Packing Instruction - the packing specifications you followed when preparing the package. For Infectious Substances, it is 620.

Additional Handling Information - important to fill in with the following information:

Emergency Contact Telephone number - This is absolutely required. A live person must answer the number 24 hours a day. Pager numbers are not acceptable.

Appendix C.8 Example of triple packaging of non-regulated biological materials



Three 15ml Falcon tubes (primary container) are placed in a high performance absorbent pouch that also acts to cushion the primary container, and are assembled into a sealable zip lock bag that acts as a secondary container. The assembled pack is then placed into a leak-proof tertiary carrier with a lid that can be fastened. An appropriate coolant can be placed inside the tertiary carrier to maintain sample integrity during transport.

Appendix D.1 Toxins and decontamination solutions

Toxin	NaOCl (30 min)	NaOH (30 min)	NaOCl + NaOH (30 min)	Ozone Treatment
Botulinum neurotoxin	> 0.1% ^a	> 0.25 N	ND	Yes ^b
Staphylococcal Enterotoxin	> 0.5% ^c	> 0.25 N	ND	ND
Ricin	> 1.0% ^d	ND	> 0.1% + 0.25N ^e	ND
Saxitoxin	≥ 0.1% ^a	ND	0.25% + 0.25N ^e	ND
Palytoxin	≥ 0.1% ^a	ND	0.25% + 0.25N ^e	ND
Microcystin	≥ 0.5% ^a	ND	0.25% + 0.25N ^e	ND
Tetrodotoxin	≥ 0.5% ^a	ND	0.25% + 0.25N ^e	ND
T-2 mycotoxin	≥ 2.5% ^f	ND	0.25% + 0.25N ^e	ND
Brevetoxin (PbTx-2)	≥ 2.5% ^f	ND	0.25% + 0.25N ^e	ND

Notes:

ND indicates “not determined” from available literature.

- Solutions of NaOCl (>0.1%) or NaOH (>0.25N) for 30 minutes inactivate *Botulinum* neurotoxin (BoNT) and are recommended for decontaminating work surfaces and spills of *C. botulinum* or BoNT. Chlorine at a concentration of 0.3-0.5 mg/L as a solution of hypochlorite rapidly inactivates BoNT (serotypes B or E tested) in water. Chlorine dioxide inactivates BoNT, but chloramine is less effective.
- Ozone (>2 mg/L) or powdered activated charcoal treatment also completely inactivates BoNT (serotypes A, B tested) in water under defined condition.
- SEB is inactivated with 0.5% hypochlorite for 10-15 minutes.
- Ricin is inactivated by a 30 minute exposure to concentrations of NaOCl ranging from 0.1-2.5%, or by a mixture of 0.25% NaOCl plus 0.25 N NaOH. In general, solutions of 1.0% NaOCl are effective for decontamination of ricin from laboratory surfaces, equipment, animal cages, or small spills.
- The minimal effective concentration of NaOCl was dependent on toxin and contact time; all LMW toxins tested were inactivated at least 99% by treatment with 2.5% NaOCl, or with a combination of 0.25% NaOCl and 0.25N NaOH.
- For T-2 mycotoxin and brevetoxin, liquid samples, accidental spills, and non-burnable waste should be soaked in 2.5% NaOCl with 0.25% N NaOH for 4 hours. Cages and bedding from animals exposed to T-2 mycotoxin or brevetoxin should be treated with 0.25% NaOCl and 0.025 N NaOH for 4 hours. Exposure for 30 minutes to 1.0% NaOCl is an effective procedure for the laboratory (working solutions, equipment, animal cages, working area and spills) for the inactivation of saxitoxin or tetrodotoxin.

Appendix D.2 Drug degradation and inactivation with potassium permanganate (KMnO₄)

Source: Benvenuto et. al., 1993

Drug	Proportion (mg KMnO ₄ / mg drug)	Reaction Time, h	Parent Drug Remaining, %	Mutagenicity after Reaction, %
Etoposide	2.42	Immediate	0	0
Teniposide	2.65	Immediate	0	0
Bleomycin sulfate	0.34 ^a	Immediate	0	0
Mitomycin C	0.91	Immediate	0	0
Methotrexate	1.04	Immediate	0	0 ^b
Cyclophosphamide	1.70	24	24.1	16
Ifosfamide	1.82	24	0	33

^a 1 mg is equivalent to 1 unit. ^b Methotrexate is nonmutagenic and the reaction products are nonmutagenic under the assay conditions.

Appendix D.3 Degradation and drug inactivation with 5.25% sodium hypochlorite (undiluted bleach)

Source: Benvenuto et. al., 1993

Drug	Proportion (mL bleach/ mg drug)	Reaction Time, h	Parent Drug Remaining, %	Mutagenicity after Reaction, %
Etoposide	20.1	Immediate	0	0
Teniposide	28.0	Immediate	0	0
Bleomycin	7.0 ^a	Immediate	0	0
Mitomycin C	3.0	Immediate	0	0
Methotrexate	3.9	Immediate	0	0 ^b

^a 1 mg is equivalent to 1 unit. ^b Methotrexate is nonmutagenic and the reaction products are nonmutagenic under the assay conditions.

Appendix D.4 Inactivation of antibiotics by autoclaving / boiling

Antibiotics	Recommendation
Beta-lactames	
Ampicillin	Autoclaved/boiled and flushed down the drain (Destroyed at autoclaving/boiling)
Carbenicillin	
Penicillin	
Aminoglycosoides	
Geneticin (G418)	Autoclaved/boiled and flushed down the drain (Destroyed at autoclaving/boiling)
Gentamycin	
Kanamycin	
Neomycin	
Streptomycin	
Others:	
Amphotericin = Fungizon	Autoclaved/boiled and flushed down the drain
Blasticidin	Submitted as chemical waste (<i>unknown properties</i>)
Ciprifloxacin	Submitted as chemical waste (<i>is NOT destroyed by autoclaving</i>)
Enrofloxacin	Submitted as chemical waste
Erytromycin	Autoclaved/boiled and flushed down the drain
Kloramfenikol	Submitted as chemical waste (<i>is NOT destroyed by autoclaving</i>)
Nalidixinsyra	Submitted as chemical waste
Puromycin	Autoclaved/boiled and flushed down the drain
Sulfadoxin	Autoclaved/boiled and flushed down the drain
Tetracyclin	Autoclaved/boiled and flushed down the drain
Vankomycin	Submitted as chemical waste and should be substituted completely!
Zeocin	Submitted as chemical waste (<i>unknown properties</i>)
Zeomycin	Submitted as chemical waste (<i>unknown properties</i>)

Source: Morgenstern R, Hallgren C. (2009). *Rekommendationer för behandling av antibiotikaavfall vid Karolinska Institutet* and Antibiotika-Fibel, Gerg Thieme Verlag, Stuttgart, 1975.