NTU SAFETY MANUAL FOR BIOLOGICAL WORK

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Written By : Tin Tun,		
Audience : Persons conducting any biological works including work with animals		

Appoved by Institution Biological Committee, NTU

Endorsed by HSE Steering Committee, NTU

Content Page

1. Introduction

This safety manual, approved by the Institutional Biosafety Committee (IBC), is part of NTU biosafety program, established to accomplish the following:

- Protect laboratory personnel and the public from exposure to infectious agents
- Prevent environmental contamination
- Comply with relevant Acts and Regulations

The safety manual provides university-wide safety procedures for the use and manipulation of biological agents as well as the containment and control of biohazards. Although the implementation of these procedures is the responsibility of the Chairs and Directors, the success depends on the combined efforts of the Principal Investigators (PI), research staff and students.

2. Committees

2.1 Institutional Biosafety Committee (IBC)

The IBC, as stipulated in the Biological Agents & Toxins Act (BATA), oversees projects requiring Biosafety Legislation Branch's and/or Genetic Modification Advisory Committee (GMAC)'s approval. To achieve this, the committee a) defines acceptable standards and practices of biological works in NTU and b) maintains a register of all biological works in NTU.

3. Roles and Responsibilities

3.1 NTU Institutional Biosafety Committee (IBC)

The IBC members shall:

- Advise NTU on all biological and related matters through the HSE Steering Committee. Work related to the use of animals for testing or involving the use of human specimens will be directed to the Institutional Animal Care & Use Committee (IACUC) and Institutional Review Board[1] (IRB) respectively by individual PI or researcher;

- Review and amend any NTU biological and related standards and guiding documents;

- Establish biological safety training programme to achieve the competencies in carrying out biological and related works, and in particular, authorized workers in BSL-3 and BSL 2+ laboratories;

- Document and approve all biological and related projects in particular those required under BATA and GMAC requirements including risk assessment, procurement, transportation, storage, manipulation and disposal. Administration of this work can be carried out through appointing other faculty members and staff to facilitate as co-ordinators.

- Ensure safety practices are consistently carried out via internal audits through Office of Health & Safety (OHS).

3.2 School Chairs and Head of Department (HOD)

The chair and HOD bears the overall responsibility of the management and the maintenance of safe practices and procedures in the school /department.

3.3 Principal Investigator

The Principal Investigator has the responsibility for the safety within the laboratory including the biological projects and / or controlled agents[2] manipulated in his / her research laboratory.

[1] IACUC and IRB are not within the scope of this manual as these are held under separate offices.
[2] *Controlled Agents – BATA Schedule 1, 2, 3 (more than 10 litres) and 5.*

3.3.1 The Principal Investigator shall:

- Conduct a review of the proposed work and to identify potential hazards and adopt appropriate procedures before the experiment (risk management);

- Ensure safe handling of biological substances in his / her laboratory;

- Ensure that all persons working in his / her lab are familiar with the relevant legal and university requirements, and are appropriately trained and informed of the risks and hazards present in his / her laboratory;

- Submit biological project for review by NTU IBC prior to work commencement, in particular, controlled agents and genetic materials which requires specific approval from Biosafety Legislation Branch, Ministry of Health or GMAC respectively;

- Provide an inventory of biological agent, including the security methodology and projects performed, to NTU IBC Secretariat (OHS);

- Investigate any incident /accident that may affect the safety and health of workers or compromise the security of the biological agents; and

- Provide necessary resources to maintain good safety practices and safe operation of the lab including assigning users in taking care of equipment such as Biosafety cabinets, autoclaves, or security of infectious agents and hazardous substances.

3.3.2. If the Principal Investigator leaves the university whilst the project is still on-going, the Principal Investigator shall inform the Secretariat for NTU IBC with the proposal of the termination or transference of the project to another person or location. The cost of disposal, transfer and clean up of the laboratory shall be borne by the PI and / or the school/department.

3.4 Persons at Work[3]

The Principal Investigator or the applicant declares name of persons involved in the funded research project when application is submitted to IBC for review and approval. All persons at work in biological laboratories must comply with all university requirements and standards that are applicable to their area of work set out in this document.

Support staff such as maintenance service personnel (i.e. operations support staff, external contractors) should have the knowledge or be informed of the nature of work of the laboratory, and of the safety regulations and procedures of the university.

3.5 Office of Health and Safety (OHS)

OHS shall, on behalf of the IBC,

- Conduct audits, routine inspections and unscheduled checks for any biological work;
- Ensure the school or department safety committee or the PI conducts investigation whenever an incident /accident had occurred and ensuring control measures have been implemented; and
- Oversee the process of approval including the review of risk assessment, storage and disposal as required in this document.

3.6 School / Deaprtment Biological Project Administrator

- Ensure that all Funded Biological Projects in the school or department have IBC's approval and BPN;
- Provide assistance in online BPN application as required;
- Keep track and document biological projects in the school or department via the online BPN application system;
- Corordinate with IBC or OHS for biosafety issues;
- Cooperate with laboratory or facility users in the school or department on biosafety measures

[3] Including any research worker and students (graduate or undergraduate).

4. Biosafety Requirements

4.1 Registration of Biological Projects

The Principal Investigator (PI) shall register all funded projects involving biological agents or microorganisms and their toxins which are defined in the relevant schedules in BATA (some microorganisms or toxins may not be listed) and GMOs by submitting a biological project application form with the approved risk assessment form to the NTU IBC via the NTU online system. Refer to **Appendix 1** for application requirements. After review and approval by the IBC a Biological Project Number (BPN) will be given. If GMOs are involved the PI must fill out GMAC Proposal Form of Genetic Manipulation Work and submit together. These will be forwarded to the respective authority for clearance after internal review by NTU IBC. The PI can only commence work after they have obtained a BPN. Any change in the scope or location of work will require a new BPN application.

For projects / tasks that do not require any grant funding (i.e. teaching activities) risk assessments are approved by the HOD and do not need the IBC's approval.

The Principal Investigator who is not NTU staff but wants to utilize NTU facility for biological project needs some kind of authorization to a NTU staff for submission of online application to the IBC for review and approval. The approval process information will be communicated to the authorized NTU staff only.

NTU PIs who work in non-NTU facilities need to notify NTU IBC for their funded biological projects . Biosafety review and approval of the project may be given by the host IBC.

All PIs are accountable for the inventory of the biohazardous agents in his / her laboratory. To this the PI shall ensure a minimum half yearly stock take of the biological inventory and a monthly inspection of the laboratories.

4.2 Biological Agents & Toxins

Biological agents and toxins capable of causing diseases in human (i.e. human pathogens) are classified into five schedules under BATA.

http://statutes.agc.gov.sg/aol/search/display/view.w3p;page=0;query=DocId%3A6b6eae33-48b3-4aeb-bbbd-651b14629c01%20Depth%3A0%20ValidTime%3A01%2F10%2F2011%20TransactionTime%3A22%2F09%2F2011%20Status%3Ainforce;rec=0;whole=yes

MOH has also adopted the Laboratory Biosafety Manual, 3rd Edition, the World Health Organisation (WHO) as the national guidelines for biosafety to supplement BATA.
http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf?ua=1

4.3 Approvals and Permits in BATA

Table 1: Controls for the 5 Schedules in BATA

Controls	Schedule 1		Schedule 2	Schedule 3	Schedule 4	Schedule 5	Inactivated Agents	
	Part I	Part II					Schedule 1	Schedule 2
Import Permit	√	√	√	√	√	√	√	√
Transhipment 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**

Approval for Import

An import permit is required for all biological agents under all Schedules. Each permit is valid only for the specific consignment if the biological agent for which the permit had been granted.

Approval to Possess

Approval to possess biological agents in Schedules 1 and 2 and toxins in Schedule 5 are agent-specific. No approval to possess is required for biological agents in Schedules 3 and 4. The approvals to possess agents in Schedule 1 (Part II) and Schedule 2 are granted to a certified BSL-3 facility officially recognized as a protected place under the Protected Areas and Protected Places Act. The approval to possess toxins in Schedule 5 is granted to a certified or uncertified facility which is officially recognized as a protected place.

Applications for Schedule 1, 2 and 5 BAs and Toxins

These applications must be endorsed by NTU IBC before submission to Ministry of Health (MOH). For Genetically Modified Organisms (GMOs), approval must be sought from NTU IBC and Genetic Modification Advisory Committee (GMAC) before submitting an application for approval and permit to MOH.

4.4 Recombinant DNA Experiments

Projects involving genetic manipulation are required to adhere to the guidelines from GMAC. These guidelines ensure that such experiments are properly regulated and supervised (containment, handling and transport of GMOs) so that they will not pose a threat to public health.

GMAC has released *"The Singapore Biosafety Guidelines for Research on Genetically Modified Organisms (GMOs)*（http://www.gmac.gov.sg/）which cover experiments that involve the construction and/or propagation of all biological entities that are either made by genetic manipulation or of a novel genotype and which are a) unlikely to occur naturally or b) could cause public health or environmental hazards. The Guidelines also covers the importation of GMOs and/or GMO-derived products for research purposes.

Notification must be made to GMAC depending on the category of experiments shown in Table 1 (See also section 5 for classes of experiments falling within each category)

Table 1: Different categories of experiments with notification criteria

Category	Description	IBC Approval	Initial GMAC Review	GMAC Notification
A	Regulated Experiments with Significant Risk	√	√	√
B	Notifiable Experiments with Low Risks	√	√	×
C	Experiments with No Significant Risks (Exempt from Guidelines)	√	√	×

PIs must fill out the GMAC 'Proposal Form for Assessment of Genetic Manipulation Work', submit a copy to GMAC via NTU IBC and obtain the relevant approval before work can be commenced.

4.5 Animals

All research experiments involving animals must be approved by and conducted in accordance with NTU Institutional Animal Care and Use Committee (IACUC) approved protocols, for more details refer to: http://research.ntu.edu.sg/guides/Pages/FormsandDocuments.aspx

4.6 Human Subjects / Human Tissues

All research projects involving the use of human subjects or human tissues must be submitted for review by NTU Institutional Review Board (IRB) on the ethical use of human subjects. Refer to the IRB website for guidelines and application
http://research.ntu.edu.sg/GuidelinesnForms/Pages/default.aspx

4.7 Training

All laboratory personnel (staff, graduate and FYP students) handling biological materials must be competent. They must pass the Basic Biosafety Training on edveNTUre prior to commencement of lab work. In addition lab workers must also take other related courses needed in the course of their work such as risk management, spill, use of PPE[4] and incident reporting.

For work with vertebrate animals, all personnel are required to undergo the "Responsible Care and Use of Laboratory Animals (RCULA)" Course.

4.8 Inspections

PIs should ensure frequent internal audit and inspections to ensure a safe laboratory working environment. A useful guide is the inspection checklists found in NTU Biosafety Laboratory Inspection Checklist.

Inspections and audits are also carried at the School or University levels by the School Safety Committee or OHS respectively.

4.9 Standard Operating Procedures (SOPs)

Standard Operating Procedures, or SOPs, are required to enable method for performing common routine tasks are done safely. SOPs are to be reviewed regularly for safety improvements.

4.10 Occupational Health Programs

Laboratory Personnel must be provided appropriate immunization.

a) Working with animals:

- with a tetanus vaccine prior to working with these animals
- booster shot every 10 years.

b) Working with materials of human origin (human blood, tissues, body fluids, cell lines etc.) of both commercial and non-commercial sources:

- with a Hepatitis B vaccine, if tested negative for Hepatitis B antibodies. Screening of antibody levels done after six months.
- Re-screening after 10 years.

[4] Personal Protective Equipment (Refer to SOP on the use of PPE).

c) Working with regulated biological agents (especially BATA schedule 1, 2 or 5):

- Appropriate medical surveillance and occupational health programmes
- Specific immunization recommended by the Designated Occupational Health Physician (DOHP).

(Refer to “SOP on Immunization Grid” and “SOP on Medical Surveillance” for more information.)

Occupational related disease, illness or infection

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

i. Affected persons at work are sent for medical assessment immediately in Accident & Emergency Units of Hospitals and/or with any healthcare professional;
(Refer to “Obtaining medical help after a hazardous laboratory exposure “ at http://www.ntu.edu.sg/ohs/workplacesafety/Pages/Laboratory.aspx)

ii. A report is to be submitted to OHS via the Incident Investigation & Reporting Form (IIRF) at http://www.ntu.edu.sg/ohs. Depending on diagnosis, reports will have to be submitted to Ministry of Manpower and/or Ministry of Health by OHS;

iii. Report is to be made to Designated Occupational Health Physician (DOHP) in NTU for follow up and review;

iv. Risk Assessment (RA) related to the project is to be reviviewed and updated.

5. Managing Biosafety

Biosafety is shared responsibility at all levels the University. The University acknowledge the institution's role in providing a safe workplace and delegate the NTU IBC the authority to administer biosafety program and the OHS to carry out / assist in the function of IBC.

The PIs and all laboratory personnel who perform work with biohazards are the most important component of the biosafety program, as they must incorporate the biosafety requirements into all facets of their work.

The school and department appoint Biological Project Administrator who look into biosafety issues for projects or works carried out in their school or research centre.

5.1 Classification of Biological Agents

Risk Group and BATA Schedules

Infectious agents are classified into risk groups based on the five schedules in BATA. The 5 schedules (Table 2) cover a spectrum of biological agents and toxins with different levels of controls adopted for each schedule. BATA differentiates between higher and lower risk groups biological agents, and those with potential to be weaponised[5].

Table 2: Description of the 5 Schedules in BATA

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

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

[5] Weaponised in this document means with a potential of being used as a biological weapon of mass destruction.

5.2 Biosafety Levels

For each Risk Group of microorganisms there is a defined minimum set of control measures known as Containment Level or Biosafety Level (BSL) that reduces exposure to an acceptable level for microorganisms of that Risk Group.

There are four biosafety levels (BSL 1 to 4). 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.

Biological laboratory facilities in NTU follow guidelines stated in WHO Laboratory Biosafety Manual. These are:

Biosafety Level 1 (BSL1)	Biosafety Level 2 (BSL2)	Biosafety Level 3 (BSL3)	Biosafety Level 4 (BSL4)
Basic	Basic	Containment	Maximum Containment
Basic teaching and research laboratories where basic biosafety rules stated in section 6 are in place.	Research laboratories with biosafety practices plus protective equipment, biohazard sign, controlled laboratory access and biological safety cabinet. (section 6 & section 7) Most of the research laboratories in NTU are classified as BSL-2	Containment laboratory with practices as Level 2 plus - special clothing - strict controlled access - directional air flow - biological safety cabinets - other primary devices for all activities This special facility is set up as a core facility for research work involving biological materials of potential biohazard.	Not available in NTU.

N.B: Bio-safety level designations are based on a composite of the design features, construction, containment facilities, equipment, practices and operational procedures for working with agents from the various risk groups.

5.3 NTU- BSL-3 Laboratory

Currently only NTU- BSL-3 laboratory (which requires annual certification) allows experiments involving Risk Group 3 or BATA Schedule 1 (part 1) biological agents.

Some of the Schedule 1 biological agents are allowed to be manipulated in a BSL2 lab, subject to written approval from MOH.

6. Laboratory Practices

Human error, poor laboratory techniques and misuse of equipment cause the majority of laboratory injuries and work-related infections.

6.1 Basic Rules of Biosafety

The Basic Rules of Biosafety of any personal laboratory work ethic are:

1. Do not mouth pipette. Use mechanical pipetting devices
2. Manipulate infectious fluids carefully to avoid spills and aerosol production.
3. Use needles, syringes and other “sharps” carefully to avoid self-inoculation; and dispose of sharps in puncture- resistant and leak-proof containers without re-capping.
4. Use personal protective equipment such as laboratory coats, gloves and eye protection.
5. Observe personal hygiene; Wash hands immediately after all laboratory activities, removal of gloves, contact with any biological materials and chemicals and just before leaving the laboratory.
6. Decontaminate work surfaces before and after use as well as immediately after spills.
7. Do not eat, drink, store food, apply cosmetics or smoke in the laboratory.
8. Maintain good housekeeping

Each laboratory personnel working with biohazardous agents must be constantly aware of the importance of the proper attitude in preventing accidents in the laboratory.

6.2 Signs and Labels

6.2.1 Biohazard Warning Sign

A biohazard label (BSL 2 / BSL 3) is required for all areas or equipment which contain biohazardous or toxic agents. The appropriate place for posting the label is at the main entrance door(s) to laboratories and animal rooms, on equipment like refrigerators, incubators, transport containers, and/or lab benches.

6.2.2 Door signs

Each laboratory must have a sign at the entrance of the room that provides safety information to visitors and service personnel.

Entrance to laboratories that handle BSL2 materials, 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 BSL2.

6.3 Selection of PPE

Use the following PPE to minimize exposure via mucous membrane OR non-intact skin:

- For face and eye protection, wear safety glasses and a mask, or chin length face shield whenever splashing, splattering or droplets may be anticipated. An impact resistant face shield should be used when operating autoclaves to protect the user’s face against splatters of hot liquids or broken glass fragments.
- Gloves and a lab coat are worn to protect the skin and clothing from contact with potentially infectious materials. Wear gloves that are long enough to extend over the sleeves of the lab coat and cover wrists. Consider double gloving when working with cultures of infectious agents or handling spills.
- Sleeve covers are worn over lab coat and gown sleeves to provide protection to the sleeves and wrists from contamination when working in the biological safety cabinet. Disposable sleeve covers have tight fitting grips at both ends.
- Waterproof bandages are worn to cover any wounds or non-intact skin before gloving. It is preferred to double glove when skin is damaged or non-intact. Inform your supervisor of any severe skin condition or wounds. Avoid working with BL2, BL3 or other potentially infectious materials if non-intact skin cannot be adequately covered.
- Solid front gowns provide more protection to clothing and skin than lab coats. Solid front gowns are worn for high hazard infectious agent work. The tight fitting cuffs of the gown help to minimize wrist contamination.
- Impervious lab coats, gowns or aprons are worn when heavy contamination or soiling is likely.
- Head covers are worn to protect the hair or scalp from splatter or droplets when working with heavy contamination or when contact with head is likely.
- Shoe covers are worn over shoes to protect shoes from contamination when working in heavily contaminated areas.
- Gowns, head and shoe covers also help keep contaminants from entering the sterile area in clean rooms or sterile environment.

6.4 Biosafety Levels (Biosafety Level 1, 2, 2+ and 3)

6.4.1 Biosafety Level 1

When you are working in BSL1,

- Keep laboratory door closed especially when experiments are in progress,
- Use procedures that minimize aerosols.
- Do not smoke, eat, drink or store food in BSL1 areas,
- Wear laboratory gowns or coats.
- Avoid using hypodermic needles.
- Disinfect work surfaces daily and immediately after a spill.
- Decontaminate all biological wastes before discard. Decontaminate other contaminated materials before washing, reuse or discard.
- For off-site decontamination, package contaminated materials in closed, durable, leakproof containers.
- Control insect and rodent infestations.
- Keep areas neat and clean.

6.4.2 Biosafety Level 2

When you are working in BSL2,

- Keep laboratory door closed.
- Post a biohazard label on equipment where infectious agents are used / stored.
- Allow only persons informed of the research to enter BSL 2 areas.
- Keep animals not used in BSL 2 experiment out of the laboratory.
- Wear PPE (laboratory gowns or coats, gloves and face and eye protection) when appropriate; do not wear PPE outside of the laboratory.
- Change PPE when soiled or compromised.
- Use procedures that minimize aerosol formation.
- Avoid using hypodermic needles.
- Substitute plastic for glass where feasible.
- Use biological safety cabinets to contain aerosol- producing equipment.
- Disinfect work surfaces daily and immediately after a spill.
- Maintain a biological spill kit within the laboratory.
- Ensure that all biomedical waste containers are labeled with the biohazard symbol.
- Decontaminate all biological wastes before discard. Decontaminate other contaminated materials before washing, reuse, or discard.

- For off-site decontamination, package contaminated materials in closed, durable, leakproof containers.
- Control insect and rodent infestations.
- Keep areas neat and clean.

6.4.3 Biosafety level 3

Biosafety Level 3 (BSL3) is applicable to work done with indigenous or exotic agents with a potential for respiratory transmission and which may cause serious and potentially lethal infection.

a. Primary hazards to personnel working with these agents include autoinoculation, ingestion and exposure to infectious aerosols.
b. 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.
c. 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.
d. Secondary barriers include controlled access to the laboratory and a specialized ventilation system that minimizes the release of infectious aerosols from the laboratory.
e. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent persons who are experienced in working with these agents.

6.4.4 Biosafety Level 2+ (BSL 2+)

Some experiments with BSL2 agents are approved only at BSL2+ containment. This means that work can be done in an ordinary BL-2 laboratory but BSL 3 work practices must be utilized.

Some existing facilities may not have features recommended for BSL 3 (i.e. double-door access zone and sealed penetrations). An acceptable level of safety for the conduct of routine procedures (i.e. diagnostic procedures involving the propagation for identification etc.) may be achieved in a Biosafety Level 2 facility provided that:

1) exhaust air from the laboratory room is discharged to the outdoors,
2) directional airflow into the laboratory is ensured.
3) there is controlled access to the laboratory when work is in progress, and
4) recommended BSL3 work practices are rigorously followed.

However, the implementation of such modifications should adhere to MOH recommendations, WHO guidelines for biosafety as well as recommendations of NTU IBC.

6.5 Packaging & Transportation of Biological Agents

6.5.1 Transportation Of Biological Materials (within and outside NTU campus)

All biological materials should be transported in a way that maintains the integrity of the material during normal transport conditions, as well as prevents any accidental release and endangerment to the public and the environment.

6.5.2 Transfer of Biological Agents between Buildings or Locations

Biological, diagnostic and clinical specimens, infectious materials need to be packaged in a sealed, leak proof primary container (e.g. tube), which is securely positioned in a secondary leak proof and closable container and preferably lockable (e.g., cooler, ice chest) containing a clearly visible biohazard symbol on the outside.

A list of contents as well as emergency information (e.g. PI's phone number) needs to be accompanying the material (e.g. attached to the cooler in a plastic pouch). Other information needed is a statement that the container contains infectious substance, name of the agent, name, address and phone numbers of both the transferring lab and transferee.

6.5.3 Transportation and Shipment off NTU Campus

The shipment of diagnostic and clinical specimens, biological products and infectious agents is regulated by national and international transportation rules. These include specific procedures for the correct packing and packaging of these materials, necessary documentation and labeling and permits.

Refer to the following documents for details regarding shipping of such materials:

i) The International Air Transportation Association (IATA）Guidance Document for Transport of Infectious Stances

ii) Biological Agents and Toxins Act

iii) Biological Agents and Toxins Act (Transportation) Regulation

iv）Multi-Agency Joint Circular on Guidelines for Research, Release and Importation of GMOs

http://www.biosafety.moh.gov.sg/home

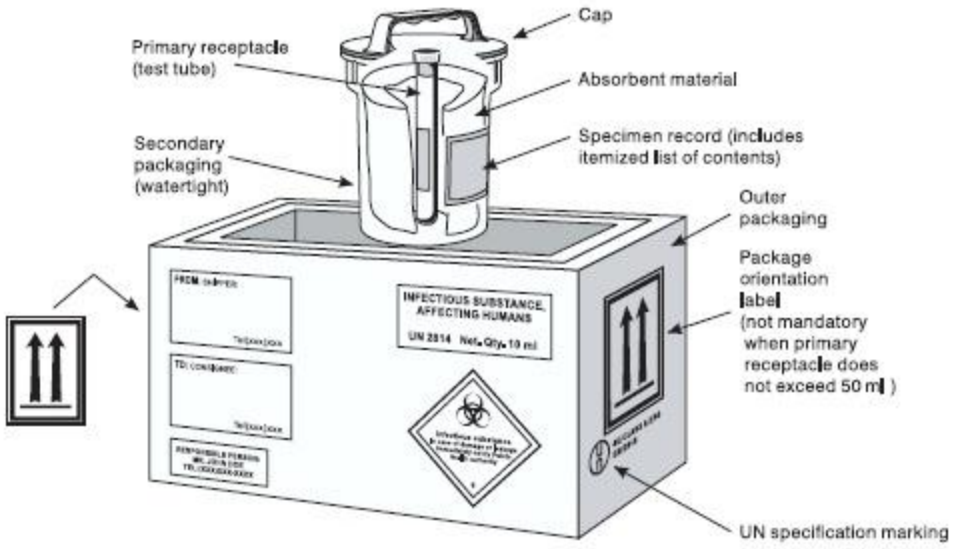
6.5.4 Types of Packaging

The types of packaging are listed under the Laboratory Biosafety Manual (World Health Organization)

6.5.5 Packing and Labeling of Category A Infectious Substances

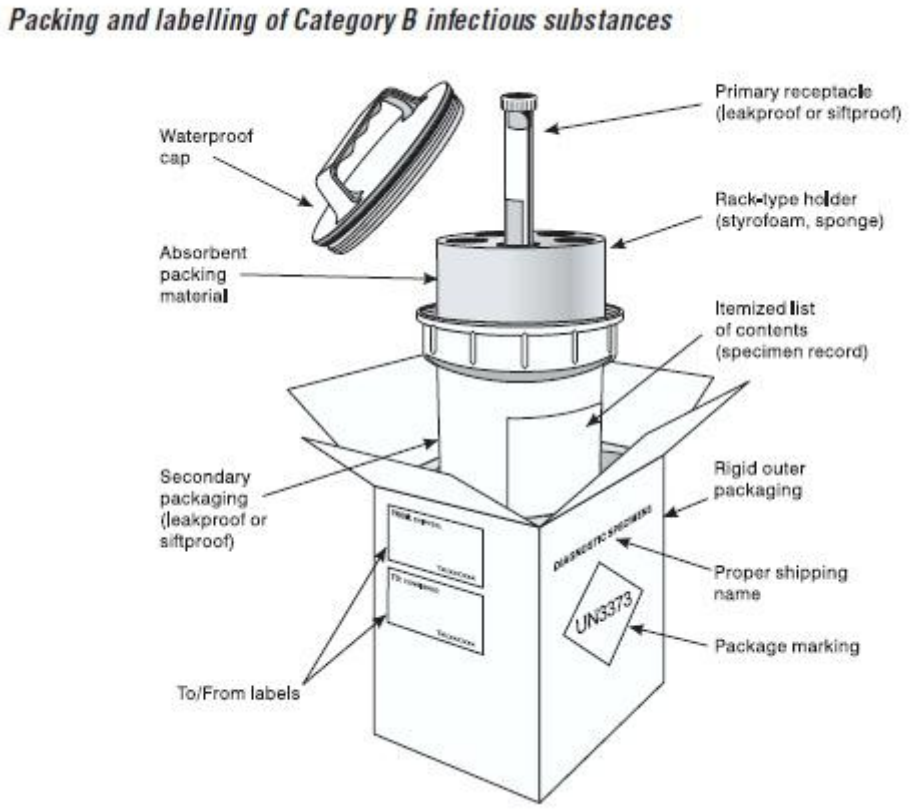
Category A material is an infectious substance that is transported in a form that is capable of causing permanent disability or life-threatening or fatal disease to otherwise healthy humans or animals when exposure to it occurs.

Packing and labelling of Category A infectious substances



6.5.6 Packing and Labeling of Category B Infectious Substances

Category B infectious substance is one that does not meet the criteria for inclusion in Category A. A Category B infectious substance does not cause permanent disability or life-threatening or fatal disease to humans or animals when exposure to it occurs.



6.6 Accidents during Transportation of Biological Agents

The BATA Transportation Regulations are to be adhered to when transporting Schedule 1, 2, 3 (quantities aggregating 10 litres or more) and Schedule 5 agents on public roads. If there is any leakage on public areas (buildings, corridors, roads, etc), users must immediately contact School Safety Officer. The School Safety Officer shall report this incident immediately to MOH Bio-safety Branch which may require reporting to SCDF Hazmat Team.

Such accidents shall be reported to NTU OHS via Incident Investigation & Reporting Form (IIRF) at http://www.ntu.edu.sg/ohs

7. Laboratory Equipment

7.1 Biological Safety Cabinets (BSC)

Biological safety cabinets (BSCs), when used properly, provide a clean work environment for research or teaching activities. The BSC provides primary containment for infectious materials and so protecting persons at work from coming into contact with these substances. However, the effectiveness of BSCs to achieve the desired containment will depends upon the behavior of the operator and the installation of the unit such as proximity to traffic flow in labs.

BSCs are designed to contain aerosols generated through the use of laminar air flow and high efficiency particulate air (HEPA) filtration. Three types of biological safety cabinets (Class I, II and III) are used in laboratories.

It is required to prohibit the use of various types of open flame inside the BSC including wick lamps, fire boys, microburners or cartridge burners.. Any form of open flame is a potential source of ignition for a fire. The heat generated disrupts the air control system of the BSC making contamination due to eddy current very likely. Additionally, the heat will may cause damage to the HEPA filter.

7.1.1 Class I Biological Safety Cabinet

- Suitable for work involving low to moderate risk agents, where there is a need for containment, but not for product protection.
- It provides protection to personnel and the environment from containments within the cabinet but does not protect the work within the cabinet from “dirty” room air.

7.1.2 Class II Biological Safety Cabinet

- protects the material being manipulated inside the cabinet (e.g. cell cultures) from external containment.
- protect personnel, the environment and the product.

There are three basic types of Class II biological safety cabinets: Type A, Type B and 100% exhaust. The major differences between the three types may be found in the percent of air that is exhausted or recirculated, and the manner in which exhaust air is removed from the work area.

7.1.3 Class III Biological Safety Cabinet (gas-tight or called glove box)

- the highest attainable level of protection to personnel, environment and product.
- provides a total physical barrier between the product and personnel.

It is for the use with high risk biological agents and is used when absolute containment of highly infectious or hazardous material is required.

Proper operation and maintenance of a BSC is essential for effective protection to be provided. BSC must be certified annually by a qualified person. The school and department shall track and put in place a maintenance regime for the use of BSC in the laboratory.

7.2 Autoclaves

Autoclaves are pressurized equipment used for heat sterilization. The autoclaving process commonly use steam heated to 121^0C, at 15 psi above atmospheric pressure. Autoclaves are generally used to sterilize instruments, media and glassware, and to decontaminate biohazardous wastes prior to disposal.

Appropriate PPE must be worn when operating autoclaves and when handling potentially infectious materials to be autoclaved.

Preventive maintenance and quality control checks must be done to ensure proper performance of the equipment. Autoclaves must be inspected by Ministry of Manpower authorized boiler inspectors yearly or bi-yearly depending on the capacity of the autoclave. A licence sticker will be issued for this upon satisfactory inspection.

7.3 Procedures for Centrifugation

All centrifugation shall be done using centrifuge safety buckets or sealed centrifuge tubes in sealed rotors. If a small centrifuge is used and centrifuge cups are not available; the centrifuge should be operated in the biological safety cabinet. Each person operating a centrifuge should be trained on proper operating procedures.

The following procedures for centrifugation are recommended:

- Keep a logbook detailing operation records for centrifuges and rotors to assist in determining service requirements.
- Examine tubes and bottles for cracks or stress marks before using them.
- Fill and decant all centrifuge tubes and bottles within the biological safety cabinet. Wipe outside of tubes with disinfectant before placing in safety cups or rotors.
- Never overfill centrifuge tubes as leakages may occur when tubes are filled to capacity. (maximum is ¾ full)
- Always cup tubes before spinning.

- Place all tubes in safety buckets or sealed rotors. Inspect the “O” ring seal of the safety bucket and the inside of safety buckets or rotors.
- Wipe exterior of tubes or bottles with disinfectant prior to loading into rotor or safety bucket.
- Never exceed safe rotor speed.
- Stop the centrifuge immediately if an unusual condition (noise or vibration) begins.
- Wait five minutes after the run before opening the centrifuge. This will allow aerosols to settle in the event of a breakdown in containment.
- Decontaminate safety carriers or rotors and centrifuge interior after each use.
- Open safety buckets or rotors in a biological safety cabinet.

7.4 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 biosafety 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 inoculums.

- 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-stopper 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.

(Refer to “NTU Guideline on handling of sharps” for more information)

7.5 Blenders, Mixers, Sonicators and Cell Disruption Equipment

Hazardous aerosols are created by most laboratory operations involving blending, mixing, stirring, grinding or disrupting biohazardous materials.

The use of a mortar and pestle can be a hazardous operation. Other devices that may create aerosols are ball mills, tissue grinders, magnetic mixers, stirrers, sonic cleaning devices, ultrasonic cell disintegrators and shakers. Adequate decontamination is essential prior to sonic cleaning due to possible aerosol generation. Whenever sonicators are used in the cleaning process (i.e. animal cage washers etc.) all items should be sterilized prior to cleaning.

The general laboratory practices required when using equipment that may generate aerosols with biohazardous materials are as follows:

- Operate blending, cell disruption, and grinding equipment in a biological safety cabinet.
- Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl. If the rotor is not leak-proof, inspect for leakage prior to operation.
- If the blender is used with infectious material place a towel moistened with an appropriate disinfectant over the top of the blender. Sterilize the device and residual contents promptly after use
- Blender bowls sometimes require supplemental cooling to prevent destruction of the bearings and to minimize thermal effects on the products
- Before opening the safety blender bowl, permit the blender to rest for at least one minute to allow settling of the aerosol cloud
- Grinding of infected tissues or materials with any open device is best done within a biological safety cabinet.

8. Biohazard Waste Management

Biological waste - which may be potentially hazardous to human health and /or the environment – will require special treatment and disposal.

1. All biohazardous wastes must be handled with gloves. Contaminated lab coats, gloves and other materials must be autoclaved prior to disposal.

2. To ensure adequacy of autoclave sterilization, autoclaves must be tested routinely with spore strips and other methods as recommended by manufacturer.

3. Free flowing biological liquid wastes (example cultures of microorganisms or tissue culture) shall not be disposed of with solid waste or discarded down the drainage system. The waste shall be contained in leak proof, rigid durable containers affixed with the biohazard symbol and GHS labelling.

4. Liquid biological wastes shall be sterilised by autoclaving or treated with an appropriate chemical disinfectant in accordance with the manufacturer's recommendations.

5. Biologically contaminated sharps shall be placed in appropriate sharps containers that are labeled "biohazard". All contaminated sharps are to be treated as infectious, decontaminated and disposed of only through licensed biohazardous waste collectors.

6. All biological wastes should be sent to NEA- licensed biohazardous waste collectors. Laboratory specimens or materials consisting of, containing, or contaminated with blood, plasma, serum, as well as inoculated media, cultures, and other potentially infectious materials, should be sterilized by autoclaving or disinfected before disposal.

7. Contaminated solid wastes such as cloth, plastic and paper items e.g. wrappers and paper towels must be put into autoclavable biohazard bags and autoclaved or sent for disposal by NEA- licensed biohardous waste collectors.

8. Animal carcasses, human tissues, organs and sharps wastes are to be disposed of by incineration or crememation through licensed waste contractors.

9. Wastes generated from the use of scheduled agents listed under the Biological Agents and Toxins Act must be sent for incineration and collected by authorized waste collector.

10. Wastes which cannot be autoclaved shall be chemically decontaminated. Chemically decontaminated wastes shall be disposed of by licensed toxic industrial waste collectors if the

chemicals found in the waste pose a hazard to handlers.

11. All infectious waste containers should be properly sealed and marked with the biohazard label. The PI or his designate shall ensure that all wastes are segregated and stored at the designated waste storage areas. The PI and his staff shall ensure good housekeeping for all biological wastes under their jurisdiction.

12. For biological wastes which have been effectively decontaminated and do not pose any public health concern, they can be disposed of as normal waste.

9. Emergency Procedure

9.1 Spill Management

The basic rules for responding to a spill are:

1. Immediately call for help.
2. Tend to the injured - Seek immediate medical assistance.
3. Don on the appropriate Personal Protective equipment.
4. Isolate the spill – evacuate the immediate spill area or the entire room in case of an aerosolizing (splashing or spraying) spill or a spill of volatile /hazardous material; prevent others from entering the spill area.
5. Contain the spill – place absorbent material around, on or in the flow path of the spilled material only if it can be done safely.
6. Recover spilled material into separate bags and treat absorbed material of similar hazard as the spilled material.
7. Proceed with cleanup- only if trained and properly equipped with PPE. Disinfect the spill area. Otherwise, wait for assistance of trained spill clean-up personnel / spill response teams.

9.2 Biological Spill Kit

A biological spill kit is an essential safety item for labs handling biological agents / materials.

A basic biological spill kit should include:

i. Concentrated disinfectant appropriate for the infectious agent handled in the lab e.g. household bleach.
ii. Spray bottle for making dilutions of disinfectant.
iii. Forceps, autoclavable broom and dust pan, or other mechanical device for handling sharps.
iv. Paper towels or other suitable absorbent.
v. Biohazard autoclavable bags for contaminated items.
vi. Utility gloves and medical examination gloves (or chemical resistant gloves).
vii. Face protection (eye wear and mask or full face shield).

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 which is readily available and maintained.

9.3 Accidents and Incidents Reporting

When there is any accident or incident involving a biological agent, the laboratory personnel of the area shall:

a) Inform the Reporting Officer, School Safety Officer or Safety Representative immediately and evacuate all individuals from the affected area, if necessary;

b) Block off the affected areas. of the accident area to post warning signs at all its entrances; ERT shall be called if the spill is extensive and is outside the confines of a laboratory.

c) Refer affected individuals for medical treatment, if required;

d) Inform the Office of Health & Safety;

e) Make arrangements to decontaminate any affected individual and the area and take all other actions necessary, to return the situation to normal; and

f) Ensure that contaminated items are removed for decontamination or disposal

A written investigation report has to be submitted to the Office of Health and Safety after the incident.

The Incident Investigation Reporting Form (IIRF) at http://www.ntu.edu.sg/ohs shall include:

a) the actions taken to rectify the incident and to minimize the possibility of any future recurrence;

b) the results of medical examinations carried out on affected individuals

Appendix 1

BPN Application Requirements

No	Research works	To Apply (Y/N)
1	To handle microorganism (bacteria, virus, fungi, parasite, etc.) listed in schedules in BATA	Y
2	To handle microorganism (bacteria, virus, fungi, parasite, etc.) not listed in schedules in BATA	Y
3	To manipulate any GMO or genetic material (GMAC category A, B & C)	Y
4	To handle microbial toxin listed in BATA schedule	Y
5	To handle microbial toxin not listed in BATA schedule	Y
6	To use test kit (inactivated), vaccine, protein, antibiotics, antibody, culture medium, etc. but no culture or isolation of any microorganism is involved	N
7	To do experiment with human object, diagnostic sample, autopsy sample but no culture or isolation of any microorganism is involved	N
8	To grow or isolate microorganism from natural, food or environmental sample such as waste water, sludge, etc.	Y
9	To test natural, food or environmental sample which may contain microorganisms, by mechanical, chemical or physical method, but no culture or isolation involved	N
10	To use tissue or cell culture for growing, replication or maintenance of virus or microorganism	Y
11	To handle tissue culture or cell for study of behaviour, histological staining and morphology, but no microorganism is involved in experiment	N
12	To use tissue or cell culture for transfection with plasmid or genetic material to produce infectious particle or pseudo-virus	Y
13	To use tissue or cell culture for testing with protein, antibody, etc. and chemical, physical, mechanical method	N

Approval / Permit Required from Authorized Agencies

No	Research works	Agency / Committee
1	To import any biological agents (microorganisms) and toxins – BATA schedule 1, 2, 3, 4 & 5	MOH & Customs
2	To possess biological agents and toxins - BATA schedule 1, 2 & 5	MOH
3	To transfer BATA schedule 1, 2 & 5	MOH
3	To possess / use zoonotic microorganism	AVA & MOH
4	To import animal and animal product – serum, blood, organ etc.	AVA & Customs
5	To use animal and insect in experiment	IACUC & AVA
6	To use human object and human sample	IRB

Some Important information for Biological Projects
(Extracted from MOH Biosafety Q&A)

- **What is the Biological Agents and Toxins Act (BATA)?**

The BATA stands for Biological Agents and Toxins Act.

It is a legislation that regulates the possession, use, import, transhipment, transfer and transportation of biological agents (BAs), inactivated BAs and toxins that are known to be hazardous to human health in Singapore.

In writing the legislation, recommendations from the National Biosafety Committee (NBC) and its Technical Working Committees (TWC), which are represented by related government agencies, research institutes, hospitals and key industry players, were taken into consideration.

The Ministry has also adopted the Laboratory Biosafety Manual, 3rd Edition, by the World Health Organization (WHO) as the national guidelines for biosafety to supplement the BATA.

- **Why are there several schedules in the Biological Agents and Toxins Act (BATA)? What are the kinds of biological agents (BAs) and toxins covered by BATA?**

The 5 Schedules in BATA cover a wide spectrum of BAs and toxins. Different levels of controls have been adopted for each Schedule. BATA differentiates between higher risk group and lower risk group BAs, and also those with the potential to be weaponised.

The table below shows a quick overview of the Schedules with the corresponding description and number of BAs for each:

Schedule	Description	Number
Schedule 1 Part I	Risk Group 3 BAs which can cause serious disease which is of high risk to the individual	55
Schedule 1 Part II	Description is the same as Schedule 1 Part I but they also have the potential to be weaponised.	23
Schedule 2	Risk group 4 BAs 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
Schedule 3	Risk Group 2 BAs that need special attention in large scale production	3
Schedule 4	All Risk Group 2 BAs (including those in Schedule 3) which cause disease in humans	250+
Schedule 5	Microbial toxins that have the potential to be weaponised.	7

- **I am a researcher working with genetically modified organisms (GMOs). Are there any specific requirements related to GMOs in the Biological Agents and toxins Act (BATA)?**

There are no specific requirements for work involving GMOs in the BATA. However, all work with GMOs should be submitted to the Genetic Modification Advisory Committee (GMAC) for formal endorsement and approval. Projects involving GMOs should also be approved by the respective Institutional Biosafety Committee.

If you are working with GMOs derived from Schedule 1 and 2 BAs, you need to comply with the requirements for the respective Schedules in the BATA. You may send an e-mail to GMAC Secretariat at info@gmac.gov.sg or visit the GMAC Website.

- **I am from a diagnostic laboratory and we have just diagnosed a Schedule 1 or 2 biological agent (BA). What should I do? Do I need to apply for an approval to possess?**

Handling of a biological agent in the course of carrying out a diagnosis is exempted from the Act, provided that you do not carry out activities beyond performing a diagnosis as defined in the Act.

Once the BA is no longer needed for diagnosis, you are required to destroy the BA or transfer it to a certified facility that has the approval to possess it. However, if you intend to keep the BA, or to carry out any activities that go beyond performing a diagnosis, you will need to apply for an approval to possess from MOH, then all provisions of the Act shall then apply.

- **If I am working with a zoonotic agent (i.e. a biological agent that is jointly controlled by AVA and MOH), how should I proceed with the application for an approval for possession?**

Researchers who wish to work with zoonotic agents should also write to AVA for approval of possession of these agents. AVA would need to inspect your facility before granting an approval for importation. Once you have received AVA's approval, you may proceed to apply for approvals under the Biological Agents and Toxins Act (BATA).

- **Are there any activities involving the biological agents (BAs) or toxins listed in the schedules of the Biological Agents and Toxins Act (BATA) that are exempted from the Act?**

Yes. The BATA would not apply to the following activities:

a. the disposal of any biological agent by a hazardous waste contractor;
b. the handling of any biological agent in the course of carrying out a diagnosis or an autopsy;
c. the collection of food samples or samples from the environment for the purpose of carrying out any laboratory analysis to determine or identify, for public health purposes, the nature of any biological agent that is present in such samples or in the environment from which such samples have been taken; or
d. the use or possession by any of the following persons of any finished cosmetic or medicinal product consisting of any Fifth Schedule toxin:
    1. any person lawfully manufacturing, supplying, selling or dispensing the finished cosmetic or medicinal product;
    2. any registered medical practitioner using the finished cosmetic or medicinal product in the course of treating another person;
    3. any person using the finished cosmetic or medicinal product for the cosmetic or medical purposes for which it is intended.

- **Do all the biological agents (BA) and toxins listed in the Schedules of the Biological Agents and Toxins Act (BATA) require permits to be imported?**

Yes, all biological agents (BAs) and toxins listed in the Schedules require import permit.

- **Do I need to apply for an import permit if the biological agent that I am importing is not in the list of biological agents (BAs)?**

There are biological agents that are not listed in the schedules that may also require an import permit.

For biological agents not in the "List of Biological Agents and Toxins" in the Biological Agents and Toxins Act (BATA), you may send an inquiry to moh_biosafety@moh.gov.sg so that we can advise you whether you need to get an import permit for your item.

- **Who is responsible for obtaining an import permit? Is it the importer or the carrier?**

  The importer (the laboratory or end-user of the biological agent or toxin) is responsible for ensuring that a valid import permit is obtained for the biological agent (BA) or toxin. Even if you engage a carrier to apply for an import permit on your behalf, you are responsible for providing the correct information needed for the import permit declaration to the carrier. You should ensure that the correct permit has been obtained from the MOH.

  You may require the carrier to fax the import permit so that you can verify the declaration details on the import permit is correct before authorizing the carrier to bring the goods into Singapore.

- **Is an import permit required for importation of diagnostic test kits?**

  In general, an import permit for test kits is not required under the Biological Agents and Toxins Act (BATA). The reason being most controls in a diagnostic kit have been inactivated. However, if it is known that the test-kits contain highly infectious biological agents that are capable of causing death, disease or other biological malfunction in a human, please inform MOH prior to its importation. Although diagnostic kits are generally not controlled by MOH, they are controlled by the Centre for Medical Device Regulation, Health Sciences Authority (HSA).

Version	Section	Details of Major Changes from Previous version	Document Curator	Effective Date	Next Review Date	Approved by
3.0	N.A.	Previous Release	Poon Yoke Yin	December 2012	April 2014	BSC
4.0	1	Committee has been changed to IBC from BSC	Tin Tun	30 April 2014	30 April 2016	IBC
	3.6	Appointment of Biological Project Administrators				
	4.1	Registration of biological projects and BPN online application system				
	4.4	Initial GMAC review requirement				
	4.10	Occupational Health Programs				
	6.5.3	Transportation and shipment off NTU campus				
	7.1	Biological Safety Cabinet (BSC) and To prohibit Use of Open Flame)				