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BIOSAFETY GUIDELINES: **CONTAINED USE ACTIVITY OF LIVING MODIFIED ORGANISM**

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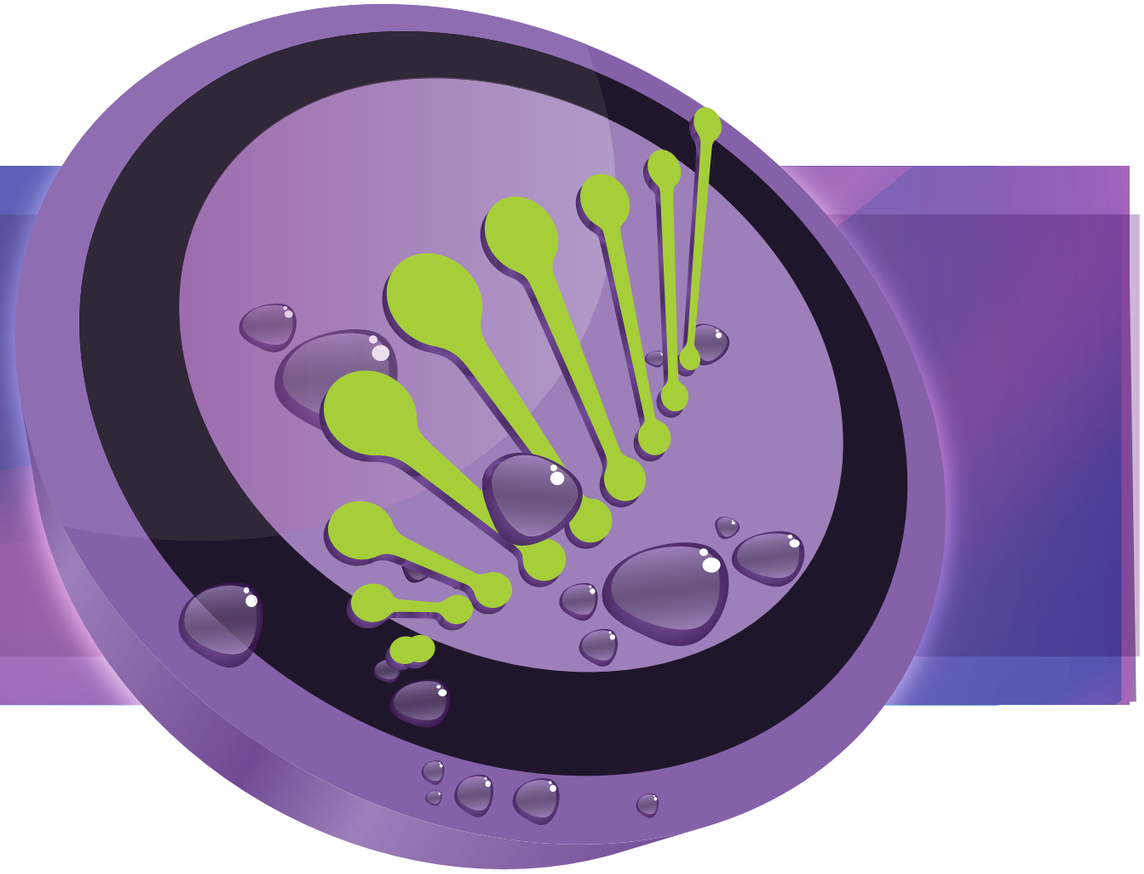

BIOSAFETY GUIDELINES FOR CONTAINED USE ACTIVITY OF LIVING MODIFIED ORGANISM (LMO)

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FORWARD

It was indeed a significant landmark event when the Malaysian Biosafety Act was passed in the Parliament on 11 July 2007 and received the Royal Ascent on 29 August 2007 before it is enforced effective from 1 December 2009. Even though it has been a rugged journey for this Act to be a reality, it is a positive and promising beginning for Malaysia to take proactive approaches towards protecting human health and the environment from the possible adverse effects of the products of modern biotechnology as well as fulfill Malaysia's obligation under the Cartagena Protocol on Biosafety. As part of the initiatives to establish legal and regulatory framework that permits effective implementation of the Act, the Ministry of Natural Resources and Environment has published the Biosafety Guidelines for Contained Use Activity of Living Modified Organism.

This guideline gives details on the Biosafety Levels (BSL) for containment as well as the safe practices for working with different types of LMO. Types of LMO outlined in the guideline are genetically modified microorganism, plant, animal, arthropod and aquatic. Other information found in this guideline are biological safety cabinet, disposal of LMO and related waste, movement, transport and storage of LMO.This guideline should be used in addition to the relevant legislations, guideline and references that involve containment facilities.

It is hoped that these guideline will be useful for all organisations who are involved in conducting research and development of modern biotechnology and to ensure that these activities comply with the Biosafety Act 2007, Biosafety (Approval and Notification) Regulations 2010 and other related government regulations and policies to safeguard human, plant and animal health and the environment.

CONTENTS

CHAPTER

1

INTRODUCTION

Recombinant DNA (rDNA) technology involves transfer of genetic material between unrelated organisms and species, creating living modified organisms (LMO). There have been concerns that such organisms might have unpredictable properties and potential harmful effects on human health, livestock, agricultural crops and the environment if there is an unintended release from the laboratory. Activitiesinvolving LMO are regulated by the *Biosafety Act 2007* and *Biosafety Regulations 2010*, to ensure safe application of biotechnology in a responsible and orderly manner.

This guideline is applicable to all research and development (R&D) activities of modern biotechnology conducted in laboratories of government and non-governmental organisations or individuals involved in such activities. Adoption of this guideline is essential for all public and private organisations, working on modern biotechnology so as to safely handle, store and transfer LMOas well as products of such organisms without endangering human, plant and animal health, the environment and biological diversity.

This guideline should be used in addition to relevant legislations and documentsas mentioned in Appendix 1.

SCOPE

CHAPTER 2

This guideline outlines:

- Biosafety Levels (BSL)
- Physical containment description
- Safe practices for working with LMO and products of such organisms

It covers the following:

a) Experiments involving LMO and products of such organisms
b) Development or production of LMO and products of such organisms
c) Breeding or propagation of LMO
d) Growth or culture of LMO
e) Import or export of LMO
f) Transport of LMO
g) Disposal of LMO and products of such organisms
h) Storage of LMO in the course of any activity mentioned in (a) to (g) above.

OBJECTIVES

CHAPTER 3

The objectives of this guideline are to:

- Identify the BSL for containment of any LMO activity
- Describe work practices under the various containment levels
- Outline the minimum requirements for setting up facilities for contained use activities of LMO
- Identify equipment requirements under the different containment levels

CHAPTER

4

RISK ASSESSMENTS

LMO and related materials are created through modern biotechnology that involves combining genetic material from different sources which produces organisms having novel genetic material or traits. Therefore, the safety of these LMO and related materials needs to be assessed and appropriate control measures implemented to limit the risk. Risk assessment is the process of estimating the potential of a hazard from an LMO activity, to give rise to an adverse outcome. The assessment is based on a combination of the likelihood of the hazard occurring and the consequences if the hazard occurs.

The classification of microorganism as in Appendix 3 does not determine the containment levels. To determine the containment levels required for LMO, the risk assessment should be done. Risk assessment should consider but is not limited to the:

- Nature of the DNA sequences that will be transferred (insert/cloned DNA) and the translated products
- Characteristics of the donor organism
- Characteristics of the recipient organism
- Characteristics of the final LMO
- Receiving environment

The characteristics of the donor, recipient and the final organism which have to be assessed (if applicable) are as follows:

- Severity of disease that may be caused
- Routes of infection
- Virulence and infectivity

- Existence of effective therapies
- Possible immunisation
- Presence/absence of transmission vectors
- Whether the agent is indigenous or exotic
- Weediness/invasiveness of the organism
- Adverse effects on the same or other species
- Any other adverse effect on biological diversity

Suitable risk management strategies should be implemented to reduce any probability of exposure to a hazard arising from activity with an LMO. Biosafety containment of the LMO activity is achieved with a combination of facility design, administrative controls, engineering controls or use of Personal Protective Equipment (PPE).

CHAPTER

5

BIOSAFETY LEVELS (BSL) FOR CONTAINMENT

There are four BSL forcontainmentbased on existing international approaches to pathogenic organisms. Theyare arranged in order of increasing stringency to reflect the level of risk involved, as indicated below:

- Biosafety Level 1 (BSL1)
- Biosafety Level 2 (BSL2)
- Biosafety Level 3 (BSL3)
- Biosafety Level 4 (BSL4)

The biosafety principles and practices described in the World Health Organisation (WHO), *Laboratory Biosafety Manual, 3rd edition, (WHO, 2004)* and the *WHO Biorisk Management: Laboratory Biosecurity Guidance (WHO, 2006)*, provide the fundamental guidelines for laboratories working with pathogenic organisms.

For genetic modification activities, five categories of containment facility are described in this guideline:

- Genetic Modification of Microorganisms (GM-BSL)
- Genetic Modification of Plants (GP-BSL)
- Genetic Modification of Animals (GA-BSL)
- Genetic Modification of Arthropods (GI-BSL)
- Genetic Modification of Aquatic Organisms (GF-BSL)

The genetic modification BSL dictates the minimum level of containment required for carrying out activities with LMO and related materials originating from these organisms. The categories of containment

facilities and corresponding work practices described in this guideline serve as a guide for any personintending to carry out contained useactivities with LMO and materials originating from these organisms. Laboratories are advised to prepare specific institutional biosafety manuals and standard operating procedures (SOP) based on the scope of LMO activities, risk assessments and risk management for specific hazards associated with their work activities.

The descriptions and assignments of containment detailed below are based on existing international approaches to pathogenic organisms. The classification of Risk Groups (RG) for organisms is listed in Appendix 2 and 3 (these lists are not exhaustive). If researchers are unsure about the containment level required for their research and development activities, they should consult their respective Institutional Biosafety Committees (IBC).

CHAPTER

6

GENETIC MODIFICATION OF MICROORGANISMS (GM-BSL)

The following guidelines are the requirements for work with genetically modified (GM) microorganisms. In addition, it is also the fundamental requirement for containment involving plants, animals, arthropods and other aquatic organisms.

6.1 Biosafety Level 1 (GM-BSL1)

General

A laboratory classified as GM-BSL1 is appropriate for conducting activities listed below:

- Research involving the introduction of naked nucleic acids into RG 1 microorganisms that has no known potential to give rise to infectious agents
- Experiments with LMO microorganisms and materials originating from these organisms of no known or minimal potential hazard to laboratory personnel, community and environment, harboring DNA from a RG 1 microorganism
- Experiments involving approved host/vector systems (refer to Appendix 4) provided that the donor DNA has no known risk to human, plant, animal, or the environment. The DNA to be introduced should be characterised fully and should not increase host or vector virulence

A GM-BSL1 laboratory is not appropriate for the following:

a) Activity with any LMO or related material that requires a higher BSL than GM-BSL1 for containment

b) The housing/keeping/rearing of any animals, arthropods or aquatic organisms for longer than the minimum time required to complete laboratory procedures on them

c) The growing of any plants except those in tissue culture or contained in a plant growth chamber

Laboratory Facility (GM-BSL1)

GM-BSL1 features include the following:

a) The laboratory is not separated from the general traffic patterns in the building

b) The laboratory is designed with open benches which can be cleaned easily

c) Bench tops should be impervious to water and resistant to acids, alkali, organic solvents and moderate heat

d) Laboratory furniture should be sturdy. Space between benches, cabinets and equipment are accessible for cleaning

e) Entrances to the laboratory should be posted with an appropriate signage identifying the type of laboratory facility and listing the procedures applicable, including emergency and maintenance procedures. The contact information of the laboratory supervisor or other responsible persons should be listed

f) Each laboratory should contain a sink for hand-washing

g) The laboratory may be equipped with fumehood

h) There should be access to a steam steriliser/autoclave in the same building

Work Practices (GM-BSL1)

a) Laboratory personnel working in a GM-BSL1 laboratory should be supervised by a scientist with general training in microbiology, molecular biology or a related science.

b) Laboratory coats should be worn during work and removed before leaving the laboratory premises. Suitable wall mounted coat hooks should be provided within the laboratory and adjacent to the access door. Personnel may be required to change into footwear specified for the laboratory (e.g. shoes covering the toes). Gloves should be worn when handling LMO and material originated from these organisms.

c) All persons handling LMO and materials originated from these organisms should wash their hands after removing gloves and before leaving the laboratory.

d) Protective eye wear should be worn for anticipated splashes of microorganisms or other hazardous materials to the face.

e) All cultures should be clearly labelled and a central logbook kept in the laboratory.

f) When experiments are in progress, access to the laboratory should be limited at the discretion of the Laboratory Supervisor.

g) Work surfaces should be decontaminated regularly and immediately after any spill of viable material.

h) All contaminated liquid or solid wastes should be decontaminated before disposal. Contaminated materials that are to be decontaminated should be placed in a durable, leak-proof container, which is closed before being removed from the laboratory. Solid wastes contaminated with rDNA materials should be decontaminated by autoclaving for at least 20 minutes at 15 psi and 121°C. A broad spectrum disinfectant is recommended for all types of surface and liquid decontamination. Reusable materials such as glass, plastic or metal equipment should be decontaminated before reuse.

i) Mechanical pipetting devices should be used, mouth pipetting is prohibited.

j) All procedures, involving liquids, are performed carefully to minimise the creation of aerosols.

k) Eating, drinking, smoking, handling contact lenses and applying cosmetics is prohibited in laboratories.

l) Food or drinks for personal consumption should not be brought into the laboratory or stored in laboratory refrigerators.

Laboratory refrigerator with NO food and drink signage

m) An insect and rodent control programme should be implemented.

Containment Equipment (GM-BSL1)

Special containment equipment is optional but generally not required for manipulation of agents assigned to GM-BSL1.

6.2 Biosafety Level 2 (GM-BSL2)

General

A laboratory classified as GM-BSL2 is appropriate for conducting activities listed below:

- Research involving the introduction of naked nucleic acids into RG 2 microorganisms that has moderate potential hazard to personnel, community and the environment
- Experiments with LMO and related materials, harboring DNA from a RG 2 microorganism.

A GM-BSL2 laboratory is not appropriate for the following:

- Activity with any LMO and related materials that requires a higher BSL than GM-BSL2 for containment
- Housing/keeping/rearing of any animals, arthropods, or aquatic organisms for longer than the minimum time required to complete laboratory procedures on them
- Growing of any plants except those in tissue culture, or contained in a plant growth chamber
- Activity with LMO cultures greater than 10 L when a large scale facility is required

Laboratory Facility (GM-BSL2)

In addition to the facility design features specified for GM-BSL1, following additional features are essential:

a) A hazard warning sign incorporating the universal biohazard symbol and the level of containment together with access restrictions should be displayed on the access door to the laboratory work area. The hazard warning sign may also identify the agent and the name and telephone number of the Laboratory Supervisor or other responsible persons.

b) The ceilings, walls and floors of the laboratory should be smooth, easy to clean, impermeable to liquids, and resistant to commonly used reagents and disinfectants. Floors should be coved to walls to facilitate cleaning.

c) A dedicated hand basin of the hands-free operation type should be provided within each laboratory, near each exit.

Hand basin facility at CSIRO

d) Windows in the laboratory should be closed and sealed.

e) An autoclave should be located within the building. Autoclaves should be certified annually by the Department of Occupational Safety and Health/DOSH (*Jabatan Keselamatan dan Kesihatan Pekerjaan*).

f) Freezers, refrigerators or other storage units used for GM-BSL2 microorganisms located outside the designated laboratory should be posted with the appropriate signage.

Laboratory ventilation(GM-BSL2)

The laboratory should be well ventilated. If required an inward flow of air should be maintained by extracting room air using mechanical ventilation to ensure directional airflow.

Large Scale Facility(GM-BSL2)

A laboratory classified as a GM-BSL2 Large Scale Facility is appropriate for the conduct of activity involving culture volumes greater than or equal to 10 L of culture of any LMO.

A GM-BSL2 Large Scale Facility is not appropriate for the following:

- activity with any LMO and related material that requires a higher BSL for containment than GM-BSL2
- the housing/keeping/rearing of any animals, arthropods or aquatic organisms for longer than the minimum time required to complete the experiments
- the growing of any plants except those in tissue culture, or contained in a plant growth chamber

Work Practices(GM-BSL2)

In addition to the work practices described in GM-BSL1, the following work practices should be observed:

a) The Laboratory Supervisor establishes policies and procedures whereby access is restricted to persons who have been advised of the potential hazard and who meet specific entry requirements (e.g. immunisation).

b) Laboratory personnel should receive appropriate training on the potential hazards associated with the work involved and the necessary precautions to prevent exposures. Personnel should receive annual updates, or additional training as necessary for procedural or policy changes.

c) Procedures which produce infectious aerosols should be conducted in biological safety cabinets or other physical containment equipment.

d) Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

e) An institutional biosafety manual is prepared or adopted for use in the laboratories. Personnel are advised on special hazards and are required to read and follow the instructions on practices and procedures.

f) Goggles, mask or face shield should be used for anticipated splashes or sprays of infectious LMO and materials originated from these materials to the face, when handling microorganism outside the biological safety cabinet.

g) Respiratory protection should be worn when aerosols cannot be safely contained.

h) All solid wastes from GM-BSL2 laboratories, including infectious wasteshould be decontaminated by autoclaving for at least 30 minutes at a pressure of 15 psi and 121 o C.

i) Liquid wastes generated during GM-BSL2 activities are to bedecontaminated immediately by mixing with a suitable disinfectant (refer to Section 12).

j) Extreme caution should be observed when handling needles and syringes to avoid accidental needle-stick injury and generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath,guard or removed from the syringe following use. Only needle-locking syringes or disposable syringe-needle units (e.g. needle is integral to the syringe) should be used for injection or aspiration of fluids from LMO or materials originating from these organisms. The needle and syringe should be promptly placed in a sharps container. Broken glassware should not be handled directly by hand, but should be removed by mechanical means, such as a brush and dustpan, tongs, or forceps, before discarding into sharps containers.

k) Spills and accidents that may cause over-exposure to LMO and materials originating from these organisms should be immediately reported to the Laboratory Supervisor and the IBC. Management of spills should follow biosafety procedures outlined in the institutional biosafety manual. An Emergency Response Plan should be documented and personnel should be trained in these procedures which include, spill management as well as incident reporting system. Medical evaluation, surveillance and treatment are provided as appropriate.

l) A record book should be maintained to provide an up-to-date inventory of the procedures performed.

Containment Equipment (GM-BSL2)

- Work with specimens containing microorganisms transmissible by the respiratory route or work producing a significant risk from aerosol production should be conducted in a Class II biological safety cabinet.

- Proper containment should be applied when procedures with a high potential for creating aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, inoculating organisms and harvesting infected tissues from organisms or other samples. A centrifuge fitted with either sealed rotors or sealed buckets should be used where large volumes or high concentrations of infectious materials are used.

6.3 Biosafety Level 3 (GM-BSL3)

General

A laboratoryclassified as GM-BSL2 is appropriate for conducting activities listed below:

- Research involving the introduction of naked nucleic acids into RG 3 microorganisms that has moderate potential hazard to personnel, community and the environment
- Experiments with LMO and related materials, harboring DNA from a RG 3 microorganism

A GM-BSL3 laboratory is not appropriate for the following:

- Activity with any LMO and related materials that requires a higher BSL than GM-BSL3 for containment
- Housing/keeping/rearing of animals, arthropods or aquatic organisms for longer than the minimum time required to complete experiments
- Growing of plants except those in tissue culture or contained in a plant growth chamber

It is recognised that some institutions intending to work with GM-BSL3 organisms, may not have all the facility safeguards recommended. In such cases, the proponent should show proof that he/she has access to GM-BSL3 facilities. Under these circumstances, acceptable safety may be achieved for routine or repetitive operations in laboratories where facility features satisfy GM-BSL2 recommendations, provided risk assessment and risk management carried out show that these procedures can be safely carried out in the GM-BSL2 facility. The proposal to implement this recommendation should be made to the National Biosafety Board (NBB) by the IBC for approval.

Laboratory Facility (GM-BSL3)

The design, construction and major changes to the GM-BSL3 facility should be certified by a competent authority/organisation endorsed by the NBB before commencement of work.

In addition to the facility design features specified for GM-BSL1 and GM-BSL2, following additional features are essential:

a) The laboratory should be physically separated from other areas including offices used by laboratory personnel and is not accessible by the general public.

b) Physical separation of the high containment laboratory from access corridors or other laboratories or activities is achieved by a double door system where entry to the laboratory facility is gained through an airlock. The airlock is provided to ensure the maintenance of the negative pressure within the GM-BSL3 laboratory and prevent airflow between the GM-BSL3 laboratory and other areas external to the laboratory. It should not be used for any work nor should it contain any equipment, washing facilities or PPE worn in the facility. Physical separation of the GM-BSL3 laboratory may also be provided by a double door clothes change room (Shower rooms may be included for personnel to shower before leaving the laboratory).

c) The laboratory and airlock should be sealable to permit safe decontamination with gases.

d) All room penetrations should be sealed to ensure they are air tight.

e) Access doors to the laboratory or containment module should be self-closing and open outwards.

f) One side of the laboratory wall should have a glass panel or observation window to view laboratory occupants from the outside. Alternatively suitable monitoring systems may be installed e.g. webcam or closed circuit television (CCTV) for this purpose.

g) An autoclave for decontaminating laboratory waste should be available within the laboratory, preferably located within the barrier wall of the GM-BSL3 laboratory, but not situated in the airlock. If located within the barrier wall, it should be accessible for maintenance from outside the laboratory.

h) Liquid effluents should be discharged in a manner appropriate to the type of waste. The method of disposal should be determined using the results of a risk assessment based on the likely composition and volume of the waste and in accordance with national regulations.

i) A telephone and fax machine or other means of outside communication should be provided inside the laboratory unit.

j) An audible emergency alarm should be provided within the laboratory to indicate a loss of negative pressure and a visible alarm is provided outside the laboratory to indicate the same.

k) The GM-BSL3 facility should be tested and certified annually at least every 12 months.

Laboratory ventilation (GM-BSL3)

The laboratory ventilation should incorporate the following features:

a) A ducted exhaust air-ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. Supply or replacement air to the room is filtered. Exhaust air should be HEPA (High Efficiency Particulate Air) filtered and discharged to the outside atmosphere in such a manner that it is dispersed away from the occupied areas and air intake passages.

b) Air supply and exhaust from Class II biological safety cabinets should be connected in a manner that avoids any interference with the air balance of the cabinets or building's exhaust system. Exhaust air from Class III biological safety cabinets should be discharged through the building exhaust system through a capture hood. It should not be re-circulated through the laboratory.

c) The proper directional airflow into the laboratory is verified by airflow tests. The laboratory should be maintained at a negative pressure of at least 50 Pa below the pressure of adjacent rooms when both doors of the airlock are closed.

Work Practices (GM-BSL3)

In addition to the work practices described in GM-BSL1 and GM-BSL2, the following work practices should be observed:

a) The laboratory senior management is required to establish policies and procedures whereby access to the laboratory is restricted to authorised persons who have been advised of the biohazard and who meet any medical requirements.

b) All personnel should have specific training in handling pathogenic organisms and in the use of safety equipment and controls. The laboratory personnel should be supervised by senior scientists who are experienced in working with pathogenic microorganisms.

c) The laboratory door should be locked when the room is unoccupied.

d) If experiments involving other organisms that require lower levels of containment are to be conducted in the same laboratory concurrently with work requiring GM-BSL3 level physical containment, such experiments should be conducted in accordance with all GM-BSL3 level practices.

e) Work surfaces of biological safety cabinets and other containment equipment should be decontaminated when work with LMO and materials originating from these organisms are concluded.

f) PPE that protects street clothing (e.g. tyvex suit and boots) and appropriate head-gear should be worn in the laboratory. In addition, appropriate respiratory protection should be made available. Personal belongings are kept in storage facilities situated adjacent to the laboratory and are not to be taken into the laboratory. If PPE is reused, it should not be worn outside the laboratory and should be decontaminated by autoclaving before being laundered.

g) Liquid wastes generated during GM-BSL3 activities should be decontaminated immediately with suitable disinfectant. All solid wastes are decontaminated by autoclaving before disposal for incineration by a government authorised service provider.

h) Laboratory animals involved in experiments requiring GM-BSL3 containment should be housed in partial containment caging systems, e.g. in open cages placed in ventilated enclosures or solid-wall and bottom cages, placed on holding racks equipped with ultraviolet irradiation lamps and reflectors that are located in a specially designated area in which all personnel are required to wear appropriate PPE providing respiratory protection.

i) A medical surveillance programme should be instituted for all persons entering a GM-BSL3 facility. When working with human pathogens, each personnel working in the laboratory should be subjected to an initial medical examination, including a relevant chest X-ray and periodic examinations. A baseline serum sample should be obtained from personnel working in the laboratory and stored for future reference. Consideration should be given to the immunisation of support personnel where appropriate.

j) An Emergency Response Plan should be documented and personnel should be trained in these procedures which include spill management, emergency evacuation if necessary, as well as incident reporting system.

Containment Equipment (GM-BSL3)

In addition to equipment specified for GM-BSL2 containment, the following should be provided:

a) Where a central reticulated vacuum system or portable vacuum pumps are used, 0.2 µm membrane-type filters and liquid disinfectant traps should be installed at the point of use.

b) Class I, Class II or Class III biological safety cabinets may be used depending on the activities.

6.4 Biosafety Level 4 (GM-BSL4)

General

A laboratoryclassified as GM-BSL2 is appropriate for conducting activities listed below:

- Research involving the introduction of naked nucleic acids into RG 4 or exotic agents that may cause serious harm to the laboratory personnel, community and environment if accidentally released
- Experiments with LMO and related materials, harboring DNA from a RG 4 microorganism

A GM-BSL4 laboratory is not appropriate for the following:

- Housing/keeping/rearing of animals, arthropods or aquatic organisms for longer than the minimum time required to complete experiments
- Growing of plants except those in tissue culture or contained in a plant growth chamber

Laboratory Facility (GM-BSL4)

The design, construction and major changes to the GM-BSL4 facility should be certified by a competent authority/organisation endorsed by the NBB before commencement of work.

In addition to the facility design features specified for GM-BSL1, GM-BSL2 and GM-BSL3 the following additional features are essential:

a) A GM-BSL4 facility is housed in a separate building.An outer and inner change-room separated by a walk-through double door shower airlock should be provided for personnel entering and leaving the facility. The outer door of the facility should be lockable.

b) Fumigation chamberor ventilated air-lock is provided for passage of materials, supplies or equipment that is not brought into the facility through the change room. Full access to all exterior surfaces of the contained structure and service penetrations should be provided to facilitate periodic integrity testing.

c) As much valve and control equipment as possible should be located outside the laboratory boundary to minimise the need for service personnel to enter the laboratory.

d) Walls, floors and ceilings of the facility should be constructed to form a sealed internal shell that facilitates fumigation and is animal and insect-proof. The internal surfaces of this shell should be resistant to liquids and chemicals, thus facilitating cleaning and decontamination

of the area. All penetrations in these structures and surfaces should be sealed.

e) If there is a central vacuum system, it should not serve areas outside the facility. In-line HEPA filters should be placed as near as practicable to each point of use. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the facility are protected by devices that prevent backflow.

f) A double-door autoclave is provided for decontaminating materials from the facility and from the inner clothing change room. The outer steriliser door opens to the area external to the facility is sealed to the outer walland controlled automatically to allow opening of the door only after the autoclave sterilisation cycle has been completed.

g) A pass through dunk tank or decontamination chamber should be provided so that materials that cannot be autoclaved can be rendered safe for removal from the facility.

h) An automatic changeover emergency power source, emergency lighting and communication system should be provided to ensure continuing operation of the ventilation systems, biological safety cabinets, room access and controls.

i) For certain requirements, a specially designed suit area may be provided within the facility. Personnel who enter this area should wear a one-piece positive pressure suit that is ventilated by a life support system that includes an alarm and emergency back-up breathing air system. Entry should be via an airlock fitted with a personal body shower into an anteroom leading to a second airlock fitted with a chemical disinfectant shower provided to decontaminate the surface of the suit before the personnel leaves the area. A double door autoclave with doors at each end is provided for decontaminating waste materials to be removed from the suit area. An air supply for connection to the positive pressure suit should be provided in the anteroom. The exhaust air should be filtered through two sets of HEPA filters installed in series. The air pressure within the suit area should be lower than that of the adjacent entry, exit and non-suit areas. A duplicate ventilation system, exhaust fan and an automatically-starting emergency power source should be provided to automatically re-establish laboratory ventilation and pressure conditions in event of equipment failure.

Laboratory Ventilation (GM-BSL4)

The laboratory ventilation should have the following features:

a) A separate supply and exhaust non re-circulating air ventilation system should be provided. The system should maintain such

pressure differentials and directional airflow towards areas of highest potential risk within the facility. There should be a differential pressure of at least 25 Pa between each area. Differential air pressure between laboratories is monitoredby use of a differential pressure gauge. The system should be provided with an alarm to detect malfunction. The supply and exhaust airflow should be interlocked to assure inward (or zero) airflow at all times.

b) Both supply and exhaust air should be filtered through HEPA filters. The HEPA filters should be installed and housed in filter chambers that are designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Within the facility, the filters are located as near the laboratories as practicable in order to reduce the length of potentially contaminated air ducts. Pre-filters to both the supply and exhaust HEPA filters should be providedto trap air supplied to the facility in order to increase the lifetime of the exhaust HEPA filters. The supply air HEPA filter should prevent the outflow of contaminated air if air pressures become imbalanced within the facility.

c) The filtered air from Class III biological safety cabinets is discharged through the facility exhaust system in a manner that avoids any interference with the air balance of the cabinets or the facility exhaust air system.

d) The ventilation control system should raise an audible alarm within the laboratory and at an attended location when room differential air pressures depart from set points.

Work Practices (GM-BSL4)

In addition to the work practices described inGM-BSL1, GM-BSL2 and GM-BSL3, the following work practices should be observed:

a) Accessibility is managed by the Laboratory Supervisor, Biological Safety Officer (BSO) or other persons responsible for the physical security of the facility. Personnel providing support services in the facility should be accompanied by authorised persons. Before entering, these persons are advised of the potential biohazards and instructed on appropriate safeguards to ensure their safety. Access to the facility should be restricted by means of secure, locked doors.

b) A facility operations manual and SOP should be prepared by the management. Practical and effective protocols for emergency situations should be documented in the SOP.

c) Authorised persons should comply with the instructions and all other applicable entry and exit procedures in the manual and SOP. All personnel entering GM-BSL4 should sign a log book and indicate the date and time of each entry and exit.

d) All personnel should be trained in the specific working aspects of the containment laboratory, including handling of the relevant pathogens, clean-up of infectious spills and use of safety equipment. Personnel are required to receive annual updates, or additional training as necessary for procedural or policy changes. Records should be maintained of all training provided.

e) A risk assessment of the working environment should be undertaken by management, taking into consideration all matters influencing the safety of personnel. Appropriate risk mitigation, monitoring and emergency response procedures should be developed and documented.

f) Personnel should enter and leave the facility through the clothing change and shower rooms, except in cases of emergency, for example fire or explosion inside the containment laboratory, personnel can use the air-locks to leave the laboratory. All street clothing including underwear should be removed and retained in the outer clothing change room. Complete laboratory clothing, including shoes should be provided by the organisation to be used by all personnel entering the facility. When leaving the facility, personnel should remove their laboratory clothing and store or discard it in the inner change room before showering. After use, the clothing is sterilised by autoclaving.

g) Supplies, materials and specimens should only be brought into the facility through the change and shower rooms, the double-door pressure steam steriliser, the fumigation chamber, the airlock or the dunk tank.

h) A primary container holding viable or intact biological material should only be opened in the maximum containment laboratory. The secondary containermay be opened in nonGM-BSL4 laboratories provided the primary and secondary containers have been decontaminated.

i) No material should be removed from GM-BSL4 laboratory unless it has been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam is decontaminated by gaseous or vapour methods in an air-lock or chamber designed for that purpose.

j) RG 4 biological materials should be stored only within the facility. If RG 4 biological materials are to be removed from the maximum containment laboratory in a viable or intact state, they shouldbe transferred in a non-breakable, sealed primary container, and then enclosed in a non-breakable, sealed secondary container, which is removed from the facility through a disinfectant dunk tank, fumigation chamber or an air-lock designed for this purpose.

k) All practices within the facility involving agents assigned to RG 4 should be conducted in Class III biological safety cabinets or alternatively Class II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life-support system.

l) Precautions should always be taken with any contaminated sharp items including needles and syringes, slides, pipettes, capillary tubes and scalpels. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as for parenteral injection, phlebotomy or aspiration of fluids from laboratory animals and diaphragm bottles.

m) A system should be set up for reporting accidents and exposures to microorganisms and for the medical surveillance of illnesses that arepotentially laboratory associated.

n) Laboratory animals involved in experiments requiring GM-BSL4 containment should be housed either in cages contained in Class III biological safety cabinets or in partial containment caging systems, e.g. in open cages placed in ventilated enclosures or solid-wall and bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors that are located in a specially designed area in which all personnel are required to wear one-piece positive pressure suits.

o) Liquid effluents including those from the shower facility, laboratory sinks, floors and autoclave chambers should be decontaminated by either heat or chemical treatment before being released from the GM-BSL4 facility. The method of disposal should be determined using the results of a risk assessment based on the likely composition and volume of the waste and in accordance with local regulations. Liquid wastes from shower rooms and toilets should be decontaminated with chemical disinfectants or by heat in the liquid waste decontamination system.

Containment Equipment (GM-BSL4)

All biological materials assigned to GM-BSL4 should be handled in Class III biological safety cabinet or Class II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life-support system.

CHAPTER

7

GENETIC MODIFICATION OF PLANTS (GP-BSL)

The following guidelines specifies physical containment and work practices suitable to conduct experiments with plants containing rDNA andplants associated with GM microorganisms, small animals or arthropods. Plant-associated microorganisms include viroids, virusoids, viruses, bacteria, fungi, protozoa and algae that have a benign or beneficial association with plants, such as certain *Rhizobium* species and microorganisms known to cause plant disease. Plant-associated small animals include arthropods that are in obligate association with plants, plant pests, plant pollinators, nematodes and those that transmit plant disease agents, for which tests of biological properties necessitate the use of plants.

The main objective of plant containment is to avoid the unintentional transmission of plant genome containing rDNA including nuclear or organelle hereditary material or release of rDNA-derived organisms associated with plants.

The following guidelines are the requirements for gene modification work with plants and describe the three biosafety levels of plant containment which falls under GP-BSL1, GP-BSL2 and GP-BSL3.

7.1 Biosafety Level 1 (GP-BSL1)

General

A facilityclassified as GP-BSL1 is appropriate for conducting activities listed below:

- Growing terrestrial plants modified with genes from other plants that have no known invasive trait
- Growing terrestrial plants modified with genes from other organisms including microorganisms that fall under RG 1

- Experiments with terrestrial plant associated GM organisms or terrestrial plant infected with these GM organisms that fall under RG 1

A GP-BSL1 facility is not appropriate for the following:

- Activity with any LMO and related material that requires a higher BSL for containment than GP-BSL1
- Activity with any LMO and related material unless they are integral to the activity being conducted in the plant facility
- Housing/keeping/rearing of any animals, arthropods or aquatic organisms unless they are integral to the activity being contained in the plant facility.

Plant Facility (GP-BSL1)

a) GP-BSL1 facilities provide the basic containment and include structures comprising greenhouses, screen houses and flexible film plastic structures.

b) The plant facility floor may be composed of gravel or other porous material. At a minimum, impervious (e.g. concrete) walkways are recommended.

c) The walls and roof should be constructed of impact resistant, transparent or translucent material to allow passage of sunlight for plant growth. Suitable materials include glass, polycarbonate and flexible film plastics such as polythene or screens.

d) Windows and other opening in the walls and roof of the facility may be open for ventilation however screens are recommended to contain or exclude pollen, microorganisms or small flying animals (e.g. arthropods and birds).

e) Entrances to the plant facility should be posted with an appropriate signage identifying the type of plant facility and listing the procedures applicable, including emergency and maintenance procedures. The contact information of the laboratory supervisor or other responsible persons should be listed.

f) All surfaces should be cleanable in accordance with the requirements for research and maintenance of healthy plants.

g) Plant facility should contain a sink for hand-washing.

Plant Containment Facility (Source: Emec Technology)

Work practices (GP-BSL1)

a) Access to the plant facility should be limited at the discretion of the Laboratory Supervisor when experiments are in progress.

b) All doors to the plant facility should be locked for the duration of the experiments except for those periods when personnel are working inside it.

c) An institutional biosafety manual describing greenhouse practices and procedures is prepared and adopted for use in the plant facility. Personnel are required to read and follow instruction prior to entering the facility.

d) Protected clothing, gloves and footwear should be worn during work and removed before leaving the plant facility.

e) Eating, drinking, smoking and the storage of food for human use is not permitted in the plant facility.

f) All persons handling the GM plants or plant associated GM organisms should wash their hands after removing gloves and before leaving the plant facility.

g) A record should be kept of experiments currently in progress in the facility.

h) Plants associated organisms which are integral to the activity should be housed in appropriate cages. If these organisms (e.g. flying arthropods or nematodes) are released within the facility, precaution should be taken to minimise escape from the plant facility.

i) Work surfaces should be cleaned daily and any spill of viable material should be decontaminated.

j) Experimental organisms should be rendered biologically inactive by appropriate methods before disposal outside of the facility.

k) A programme should be implemented to control undesired species (e.g. weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with national regulations.

7.2 Biosafety Level 2 (GP-BSL2)

General

A facility classified as a GP-BSL2 is appropriate for conducting activities listed below:

- Growing terrestrial plants modified with genes from other organisms including microorganisms that fall under RG 2
- Experiments with terrestrial plant associated GM organisms or terrestrial plant infected with these GM organisms that fall under RG 2

A GP-BSL2 facility is not appropriate for the following:

- Activity with any LMO and related material that requires a higher BSL for containment than GP-BSL2
- Activity with any LMO and related material unless they are integral to the activity being conducted in the plant facility
- Housing/keeping/rearing of any animals, arthropods or aquatic organisms unless they are integral to the activity being contained in the plant facility.

Plant Facility (GP-BSL2)

In addition to the facility design features specified for GP-BSL1, following additional features are essential:

a) The plant facility should have a floor of concrete or some other substance approved by IBC. In circumstances where the experiment requires the expressed gene to be studied at a later stage of growth (such as transgenic plant manipulated for biochemical/physiological

changes that can only be observed in the fruit/wood), the requirement for impervious floor can be omitted and the transgenic plant can be planted directly on soil.

Note: applicable only to palmae (e.g. oil palm), coconut and forest trees.

b) Any openings in the walls or roof (e.g. windows, vents, and air supply and exhaust inlets and outlets) should be screened with fine screens (thirty–gauge 30/32 mesh wire gauze). The drainage exits should be designed to avoid entry of invertebrates, rodents and insects.

c) Transparent sections of the plant facility should be made of impact-resistant material selected to maintain the integrity of the structure during all foreseeable impact events.

d) If intake fans are used, measures should be taken to minimise the ingress of arthropods. Louvers shall be constructed such that they can only be opened when the fan is in operation.

e) If the plant facility is an isolated unit, it should have an anteroom for entry and exit. The anteroom should be fitted with a sticky pest strip or electric insect-control unit to kill arthropods which gain entry. An anteroom is not necessary if the plant facility connects directly with a certified small or large scale containment facility.

f) A dedicated hand basin of the hands-free operation type should be provided near the exit within the plant facility. Where a laboratory is directly connected to the plant facility, the hand basin may be in the laboratory.

g) A liquid waste treatment system should be provided to treat all liquid waste to minimise the risk of escape of viable plant material and microorganism and ensure that the effluent is safe to be discharged.

Work Practices (GP-BSL2)

In addition to the work practices described in GP-BSL1, the following work practices should be observed:

a) The plant facility should be inspected regularly to ensure that all containment features are intact. Screens and filters should be cleaned regularly (in accordance with manufacturer's specifications).

b) Living plants or tissues should not be taken out from the plant facility except when they are being transferred to another containment facility or to an approved release site. Plants and tissues taken into or out of the facility should be carried in covered containers (refer to Section 16).

c) All plants in the plant facility should be treated as LMO. Therefore, any waste plants, tissues, soil, soil substitutes and the containers should be decontaminated before disposal (refer to Section 12).

d) The experimental materials should be inspected regularly for signs of plant pest infestation. The inspection regimen should pay particular attention to mites as they would not normally be excluded by the window and vent screens.

e) If the work permits it, plants should be sprayed regularly with a systemic insecticide or fumigated to kill other arthropods (especially mites) at regular intervals and at the end of each series of experiments.

7.3 Biosafety Level 3 (GP-BSL3)

General

A facilityclassified as a GP-BSL3 is appropriate for the activities listed below:

- Growing terrestrial plants modified with genes from other organisms including microorganisms that fall under RG 3
- Experiments with terrestrial plant associated GM organisms or terrestrial plant infected with these GM organisms that fall under RG 3

A GP-BSL 3 is not appropriate for the following:

- Activity with any LMO and related material that requires a higher BSL for containment than GP-BSL3
- Activity with any LMO and related material, unless they are integral to the activity being contained in the plant facility
- Housing/keeping/rearing of any animals, arthropods, or aquatic organisms, unless they are integral to the activity being contained in the plant facility

Plant Facility (GP-BSL3)

Certain experiments may warrant the use of plant facilities with additional containment features and operating procedures higher than those of GP-BSL2. Each project will be considered on an individual basis following a risk assessment done by IBC. Additional operating procedures within a GP-BSL2 plant facility may be recommended, instead of conducting the project in a GP-BSL3 facility.

The design, construction and major changes to the GP-BSL3 facility should be certified by a competent authority/organisation endorsed by the NBB before commencement of work.

In addition to the facility design features specified for GP-BSL1 and GP-BSL2, the following additional features are essential:

a) A hazard warning sign incorporating the universal biohazard symbol and the level of containment together with access restrictions should be displayed on the access door to the laboratory work area. The hazard warning sign may also identify the agent, and the name and telephone number of the Laboratory Supervisor or other responsible persons.

b) The facility should be constructed with a rigid reinforced frame with walls, floors and glazing forming a shell. Floors should be slip resistant. Transparent section should be made of impact resistant material such as methyl-acrylate (Perspex) or reinforce glass. Additional protection such as physical screen should be provided bare required to protect against extreme situation (storm, wild animals)

c) Joints between any structural components should be sealed and mechanically strong and durable.

d) The facility should have an anteroom/airlock for entry and exit with both doors fitted with self closing devices. The anteroom should allow materials, equipment, trolleys to pass through ensuring one door can be closed at all time. The facility should be provided with a footbath containing a suitable disinfectant.

e) Doors apart from those to areas used for showering and changing should contain glass viewing panel. Alternatively suitable monitoring systems may be installed e.g. webcam or CCTV for this purpose.

f) GP-BSL3 door signs should be posted on access doors.

g) The layout within the facility should promote the movement of ventilation air from clean site of the facility near the entry and toward the more contaminated zone such as biological safety cabinet.

h) An autoclave for decontamination of plant facility waste should be provided in the laboratory preferably located in the barrier wall of the GP-BSL3 but not located in the anteroom. If located within the barrier wall, it should be accessible for maintenance from outside the laboratory. For larger plants and trees, disinfectant dunk tank can be used for decontamination.

i) Where propagules (such as seeds, pollen or arthropod life stages) could potentially survive extended emersion under water, liquid waste outlet should be fitted with strainers or adequate fine mesh/ gauge to prevent escape. The floor of the facility should be designed such that all effluents are collected, treated and drained appropriately.

j) The facility should have a high level of physical security including barrier, fencing and restricted access provided by a controlled access system e.g. electronic access card.

k) A telephone and fax machine or other means of outside communication should be provided inside the facility.

l) The GP-BSL3 facility should be tested and certified annually at least every 12 months.

Plant Facility Ventilation (GP-BSL3)

a) A ventilation system that establishes a negative pressure in a plant facility should be provided so that there is a directional airflow into the working area. The proper directional airflow into the facility should be verified by airflow test. Air may be re-circulated within the facility. Air supply and air exhaust ducts should be fitted with HEPA filters.

b) The GP-BSL3 should be maintained at a negative pressure of at least 50 Pa below the pressure of adjacent rooms when both doors of the airlock are closed.

c) An audible emergency alarm should be provided within the facility to indicate a loss of negative pressure and a visible alarm is provided outside the facility to indicate the same.

Work Practices (GP-BSL3)

In addition to the work practices described in GP-BSL1 and GP-BSL2, the following work practices should be observed:

a) The facility senior management is required to establish policies and procedures whereby access to the facility is restricted to authorised persons. Personnel providing support services in the facility should be accompanied by authorised persons. Before entering, these persons are instructed on appropriate safeguards to ensure their safety. Access to the facility should be restricted by means of secure and locked doors.

b) A facility operations manual and SOP should be prepared by the management. Authorised persons should comply with the instructions and all other applicable entry and exit procedures in the manual and SOP. All personnel entering GP-BSL3 should sign a log book and indicate the date and time of each entry and exit.

c) Containers holding GM plants or plants associated with GM organisms should be opened only in the GP-BSL3 facility. Containers and packaging material should be decontaminated as soon as possible.

d) Personnel should wear overshoes or dedicated footwear and step into the footbath on entry and exit. Personnel should wear disposable glove and full coverage protective clothing including hair covering. Before personnel leave the facility, the PPE should be removed and placed into sealed containers for decontamination. Hands and face should be washed before leaving the facility.

e) Materials and equipment taken into or out of the plant facility should be treated by an appropriate technique to destroy or remove all other organisms (including all stages of its life-cycle). This requirement applies to soil substitutes and where feasible to soil. Soil substitutes which can be readily decontaminated should be used whenever possible.

f) A system is established for the reporting of accidents, incidents, exposures and for the medical surveillance of potential laboratory associated illnesses.

Rules and waste handling procedures pasted on the Entrance to the plant house facilitiesat CSIRO

GENETIC MODIFICATION OF ANIMALS (GA-BSL)

CHAPTER 8

The following guidelines specify physical containment and work practices suitable to conduct experiments with GM animals and animals infected with GM organisms. Animals under experiment may be either small laboratory animals (e.g. mice, rabbits) or large domestic animals (e.g. pigs, sheep, cattle). The main objective of animal containment is to prevent zoonotic infection transmitted by animals under investigation, cross infections between experimental animals, unintentional release of GM organisms from these animals into the environment and preventing escape of the experimental animals.

GM work with animals include:

- Introduction of foreign DNA into the fertilised oocyte or zygote or early embryo, or which may be performed, in or involve whole animals
- Introduction of a fragment of the whole genome or a virus into an embryo to produce a GM animal secreting infectious viral particles
- Use of GM microorganisms to infect animals

Animal facilities and work practices should be consistent with good animal welfare practices in accordance with Malaysian guidelines such as *Principles and Guide to Ethical Use of Laboratory Animals (Ministry of Health,2000)*.

The following guidelines are the requirements for genetic modification work with animals and describe the four biosafety levels of animal containment which falls under GA-BSL1, GA-BSL2, GA-BSL3 and GA-BSL4. The requirements may differ in scale as a result but the overall principles that apply are the same.

8.1 Biosafety Level 1 (GA-BSL1)

General

A facility classified as GA-BSL1 is appropriate for conducting activities listed below:

- Breeding and housing of knock-out rodents
- Breeding and housing GM rodents from one strain (propagation/ colony maintenance) infected with pathogens in RG1
- Breeding of GM animals, other than rodents, transformed with sequences of viral vector under RG 1 which do not lead to transmissible infection

A GA-BSL1 is not appropriate for the following:

- Activity with any GM animal that requires a higher BSL for containment than GA-BSL1
- Activity with any LMO and related material unless they are integral to the activity being conducted in the animal facility
- Housing/keeping/rearing any arthropods or aquatic organisms (including amphibians) unless they are integral to the activity being contained in the animal facility.

Animal Facility (GA-BSL1)

a) Facilities for laboratory and experimental animals should be physically separated from other activities such as animal breeding, housing, experiments, post-mortem examinations, quarantine, disposal of wastes and associated maintenance.

b) Entrances to the animal facility should be posted with an appropriate signage identifying the type of animal facility and listing the procedures applicable, including emergency and maintenance procedures. The contact information of the laboratory supervisor or other responsible persons should be listed.

c) All fencing, housing and handling facilities should be of a construction suitable for the secure containment of the relevant species. The fencing should be secured against escape or invasion by predatory or infectious animals.

d) A secure perimeter fence, additional to fences or housing directly containing the animals should be provided so that animals are effectively held within double fencing or housed animals are within a fenced compound.

e) Suitable handling and restraint facilities should be enclosed within the secure perimeter fence.

f) Dissection tables used in post mortem examination should be of impervious, washable material.

g) Animal facility should contain a sink for hand-washing.

h) Backflow prevention should be provided for water supplies in the facility.

Work practices (GA-BSL1)

a) Access to the animal facility should be restricted to authorised personnel.

b) All doors to the animal facility should be locked for the duration of the experiment except for those periods when personnel are actually working inside it.

c) The external perimeter fence should be checked at least every three months and after storms for any breaks or holes in the fence. Any breach should be repaired immediately.

d) Other provisions such as feed and water supplies and regular inspections should meet requirements for animal husbandry and welfare purposes.

e) All internal areas should be protected to prevent infestation by vermin. All areas should be regularly cleaned.

f) Personnel handling infected animals should be trained in safe handling procedures for the animal species involved including appropriate restraint procedures and understand the nature and hazards of the infectious agent and how it may be transmitted.

g) Eating, drinking, smoking and the storage of food for human use is not permitted in animal rooms.

h) Protective clothing, gloves and footwear should be worn in the animal facility. Protection should be taken against scratches or bites when working with animals. Hands should be washed after removing gloves and before leaving the animal facility.

i) The animals which are GM should be contained in primary containment devices such as ventilated cages fitted with exhaust HEPA filters or alternatively the animal room can be the primary containment device.

j) Cages should be labelled to indicate the identity and date of any *inocula* given.

k) Animals should be properly identified (e.g. by tattooing, microchip or permanent branding) and accounting procedures should be established.

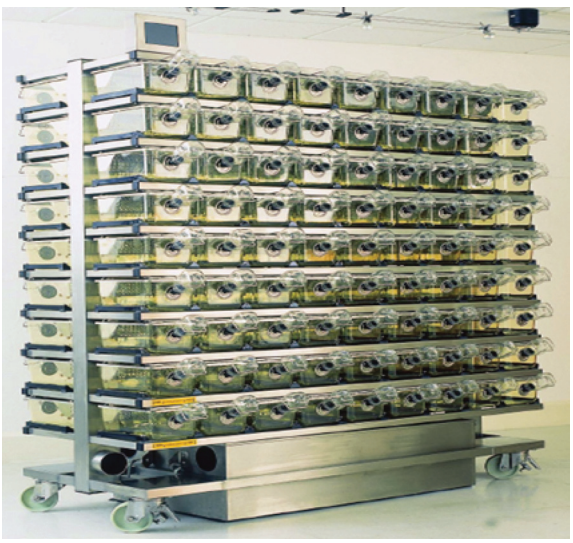
l) Exposure to animals or animal products (dander, hair or urine components) can cause allergies and asthma. Incidents of allergy should be reduced through adequate ventilation in the facility and providing local exhaust system when necessary. Personnel experiencing any unusual reaction and allergy to animals or animal products should report to the management so that appropriate action can be taken.

m) Animal carcasses which are LMO should be rendered safe by decontamination in an autoclave or incinerated and disposed of appropriately (refer to Section 12).

n) Cages, used instruments and containers should be decontaminated preferably by autoclaving before cleaning. Infectious small animal bedding and cage wastes should be rendered safe prior to disposal or reuse.

o) Bedding material and waste from animal cages or pens used to maintain animals infected with LMO should be removed in such a manner as to minimise the creation of aerosols. This material should be autoclaved before removal from the animal containment unit.

p) A programme should be implemented to control undesired species (e.g. rodent, arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with national regulations.



Individual ventilated cage (Source: Emec Technology)

8.2 Biosafety Level 2 (GA-BSL2)

General

A facility classified as a GA-BSL2 is appropriate for conducting activities listed below:

- Housing/keeping/rearing GM animals that fall under GA-BSL2
- Experiments with animal infected with GM pathogens that fall under RG 2

A GA-BSL2 is not appropriate for the following:

- Activity with any GM animal that requires a higher BSL for containment than GA-BSL2;
- Activity with any LMO and related material unless they are integral to the activity being conducted in the animal facility;
- Housing/keeping/rearing any arthropods or aquatic organisms (including amphibians) unless they are integral to the activity being contained in the animal facility.

Animal Facility (GA-BSL2)

In addition to the facility design features specified for GA-BSL1, the following additional features are essential:

a) Windows in the animal containment facility should be closed and sealed.

b) Any openings in the walls or roof (e.g. windows, vents, air supply, exhaust inlets and outlets) should be screened with fine screens (having apertures of sufficiently small gauge to prevent entry or egress of invertebrates. The mesh should be stainless steel or a suitable material).

c) Doors to animal containment facilities should open inwards to minimise the possibility of any animals escaping and be self-closing.

d) A dedicated hand basin of the hands-free operation type should be provided near the exit within the animal facility.

e) Drainage existing in the floor should contain disinfectant in the trap.

f) The animal facility should be inspected regularly to ensure all containment features are intact.

Animal Facility Ventilation(GA-BSL2)

- The animal facility GA-BSL2 should be well ventilated. If required an inward flow of air should be maintained by extracting room air using mechanical ventilation to ensure directional airflow.
- The layout within the facility should promote the movement of ventilation air from clean site of the facility near the entry and toward the more contaminated zone such as biological safety cabinet.

Work Practices(GA-BSL2)

In addition to the work practices described in GA-BSL1, the following work practices should be observed:

a) The facility senior management is required to establish policies and procedures whereby access to the facility is restricted to authorised persons. Personnel providing support services in the facilityare instructed on appropriate safeguards to ensure their safety. Access to the facility should be restricted by means of secure and locked doors.

b) An institutional biosafety manual describing animal facility practices and procedures is prepared and adopted for use in the animal facility. Personnel are required to read and follow instruction prior to entering the facility.

c) All personnel entering GA-BSL2 should sign a log book and indicate the date and time of each entry and exit.

d) Personal clothing should be covered by a laboratory coat or overalls. Closed footwear should be worn, preferably separate shoes or boots that remain within the facility. Gloves should be worn at all times.

e) PPE appropriate for the work being carried out should be worn. Respiratory protection should be used if there is a danger of infection by the respiratory route. PPE should be removed and left in the facility before leaving to other areas.

f) Work surfaces should be decontaminated after use and after any spill of infectious material.

g) For manipulations with small animals that could result in an aerosol containing viable organisms, a biological safety cabinet or other equipment designed to contain the aerosol should be used.

h) Effective disinfectants should be available for immediate use.

i) Animals involved in genetic modification experiments and tissues from these animals are not to be used for other purposes.

j) Cultures, tissues or specimens of body fluids should be placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping. Live animals or animal tissue taken from the animal facility should only go to another containment facility.

k) Animal rooms should be cleaned and decontaminated after use.

l) If experiments conducted involve arthropods or aquatic vertebrates, the following additional conditions apply:

- A mechanism should be provided to ensure that neither the organisms nor their gametes can escape into the supply or discharge system of the rearing container (e.g. tank or aquarium). The top of the rearing container should be covered to avoid escape of organisms and their gametes.
- In the case of arthropods that crawl, jump or fly, GM or non-GM animals should be kept apart.
- Measures should be taken to enable escaped arthropods to be detected and recaptured or destroyed. For ticks and mites, containers should be kept over trays filled with oil.
- All experimental cages should be numbered and documented.
- Used culture vessels should be decontaminated before disposal or thoroughly cleaned before reuse.
- Flying or crawling arthropods should be handled on white trays to facilitate the detection of escape.
- The use of an electric insect-control unit should be considered.
- The activity of arthropods and the risk of accidental escape can be reducedby chilling.
- It should be borne in mind that arthropods and aquatic vertebrates can harbour infectious organisms, for example viruses and parasites (in mosquitoes). Such arthropods and aquatic vertebrates should be kept at a containment level appropriate to the risk from the human pathogens carried by them.

8.3 Biosafety Level 3 (GA-BSL3)

General

A facility classified as a GA-BSL3 is appropriate for conducting activities listed below:

- Housing/keeping/rearing GM animals that fall under GA-BSL3

- Experiments with animal infected with GM pathogens that fall under RG 3

A GA-BSL3 is not appropriate for the following:

- Activitywith any GM animal that requires a higher BSL for containment than GA-BSL3
- Activity with any LMO and related material unless they are integral to the activity being conducted in the animal facility
- Housing/keeping/rearing any arthropods or aquatic organisms (including amphibians) unless they are integral to the activity being contained in the animal facility

Animal Facility (GA-BSL3)

The design of an animal facility is complex and those planning and construction should seek specialised advice. The design, construction and major changes to the GA-BSL3 facility should be certified by a competent authority/organisation approved by the NBB before commencement of work.

In addition to the facility design features specified for GP-BSL1 and GP-BSL2, the following additional features are essential:

a) A hazard warning sign incorporating the universal biohazard symbol and the level of containment together with access restrictions should be displayed on the access door to the laboratory work area. The hazard warning sign may also identify the agent and the name and telephone number of the Laboratory Supervisor or other responsible persons.

b) The GA-BSL3 facility should be isolated from other non-BSL3 area by an anteroom by having two doors in series with both doors fitted with self-closing devices. Where the facility forms the primary containment, an outer and inner change-room separated by a walk-through double door shower airlock should be provided for personnel entering and leaving the facility. The outer door of the facility should be lockable.

c) GA-BSL3 door signs should be posted on access doors.

d) The animal facility unit in the facility should be sealable to permit fumigation. It should be possible to decontaminate the GA-BSL3 area independently with formal dehyde gas and for the gas to be discharged safely to the atmosphere upon completion.

e) The facility should be constructed so that the finishers on walls, floors, ceiling and bench top surfaces are impervious, easily cleanable and resistant to chemicals used for gaseous decontamination.

f) If floor drains are present in the facility, it should be designed such that all effluents are collected and prior to disposal all effluents should be decontaminated by either heat or chemical treatment and discharged appropriately.

g) An autoclave for decontamination of animal facility waste should be provided preferably located in the barrier wall but not located in the anteroom. If located within the barrier wall, it should be accessible for maintenance from outside the facility.

h) Infected animals may be housed in isolators, safety cabinets or containment units.

i) The facility should have a high level of physical security and restricted access provided by a controlled access system e.g. electronic access card.

j) A telephone and fax machine or other means of outside communication should be provided inside the GA-BSL3 facility.

k) The GA-BSL3 facility should be tested and certified annually at least every 12 months.

Animal Facility Ventilation (GA-BSL3)

- A ducted exhaust air-ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. Supply or replacement air to the room is filtered. Exhaust air should be HEPA filtered and discharged to the outside atmosphere in such a manner that it is dispersed away from the occupied areas and air intake passages.

- The GA-BSL3 should be maintained at a negative pressure of at least 50 Pa below the pressure of adjacent rooms when both doors of the airlock are closed.

- An audible emergency alarm should be provided within the facility to indicate a loss of negative pressure and a visible alarm is provided outside the facility to indicate the same.

Work Practices (GA-BSL3)

In addition to the work practices described in GA-BSL1 and GA-BSL2, the following work practices should be observed:

a) Personnel providing support services in the facility should be accompanied by authorised persons.

b) A facility operations manual and SOP should be prepared by the management. Authorised persons should comply with the instructions and all other applicable entry and exit procedures in the manual and SOP.

c) No one should enter the facility for cleaning, servicing of equipment, repairs or other activities before the potentially contaminated surfaces have been disinfected and authorisation has been obtained from the laboratory supervisor or the safety officer. Dedicated cleaning equipment should be stored within the facility.

d) If the animal facility does not form the primary containment measure, all animal handling procedures with RG 3 infective materials should be done in a Class II biological safety cabinet.

e) Discarded PPE should be decontaminated preferably by autoclaving before being laundered. Where the animal facility forms the primary containment measure, a full body shower should be taken upon exiting the facility.

f) Microbiological wastes, animal excrement, animal bedding, small animal cages and animal carcasses should be rendered safe preferably by decontamination in an autoclave.

g) Animal carcasses should be rendered safe by decontamination in an autoclave followed by incineration or by alkaline digestion (refer to Section 12).

h) An effective emergency evacuation plan should be devised and information on the plan should be available to all facility personnel and local emergency services.

i) A system is established for the reporting of accidents, incidents, exposures and for the medical surveillance of potential laboratory associated illnesses.

j) Policies for the safe handling of sharps are instituted. Needles and syringes or other sharp instruments are restricted in the animal facility for use only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

k) Extreme caution should be observed when handling needles and syringes to avoid accidental needle-stick injury and generation of aerosols during use and disposal. Only needle-locking syringes or disposable syringe-needle units should be used when needed. Plastic ware should be substituted for glassware whenever possible.

8.4 Biosafety Level 4 (GA-BSL4)

General

A facility classified as a GA-BSL4 Animal Facility is appropriate for activities listed below:

- Housing/keeping/rearing GM animals that fall under GA-BSL4
- Experiments with animal infected with GM pathogens that fall under RG 4

A GA-BSL4 Animal Facility is not appropriate for the following:

- Activity with any LMO and related material unless they are integral to the activity being conducted in the animal facility
- Housing/keeping/rearing any arthropods or aquatic organisms (including amphibians) unless they are integral to the activity being contained in the animal facility.

The GA-BSL4 Animal Facility and associated work practices is complex and might need to comply with stringent requirements. Those planning to be involved in GA-BSL4 activities should refer to NBB.

CHAPTER

9

GENETIC MODIFICATION OF ARTHROPOD (GI-BSL)

The following guidelines apply to GM work with terrestrial arthropods (e.g. insects and spiders/mites) and describe the arthropod handling practices, safety equipment and facilities appropriate for Arthropod Containment Levels. The diversity of these organisms and their complex life cycles often mean that procedures and practices to safely contain the arthropods are species specific. All life-cycle stages, eggs, larvae, nymphs, adults should be handled within the arthropod containment facility. Careful consideration should be given to the design of the arthropod containment facility due to the small size, highly motile characteristics of some arthropods (especially flying and jumping) and relative long life and resistance of some stages.

Many arthropods are associated with potential risks should they escape since many are vectors of infectious human diseases. When they are experimentally infected with a human pathogen, the arthropods represent an immediate risk to those who come into contact with them. Even when they are uninfected, they can represent a risk to the community. If by escaping, they become the crucial link completing the transmission cycle for a disease they vector. The flying, crawling, burrowing, and reclusive habits of arthropods, combined with the agents they may carry, introduce an element of risk increasing behavior. The principles of risk assessment should be applied when considering containment levels. Two fates of arthropods upon accidental escape which should be considered in risk assessments are:

- Inviable- conditions are sufficiently unfavorable to the transgenic arthropod that reproduction does not occur.
- Establishment and persistence- the conditions found in the range of the arthropod species are sufficiently similar to those of the laboratory location that escaped transgenic arthropods could reasonably be expected to persist throughout the year in the local environment.

Protocols for vertebrate animals used as hosts in arthropod experiments should adhere to Malaysian guidelines such as *Principles and Guide to Ethical Use of Laboratory Animals, Ministry of Health, 2000*.

Animals which are not an integral part of the experiments and not necessary for culture of the arthropods should not be housed in the facility. Animals used as hosts or blood sources may be housed within the insectary but are adequately protected from access by escaped arthropods. The blood source is considered as a source of inadvertent arthropod infection and transmission. Measures are implemented to prevent such an event. Use of sterile blood or blood from sources known to be pathogen free is recommended. In contrast, use of blood from animals whose disease status is uncertain is to be avoided.

The following guidelines for arthropod facility and operating procedures are regarded as a minimum requirement for genetic modification work with arthropods which falls under GI-BSL1, GI-BSL2, GI-BSL3.

9.1 Biosafety Level 1 (GI-BSL1)

General

A facility classified as GA-BSL1 is appropriate for conducting activities listed below:

- Experiments with terrestrial arthropods that are modified with genes from other organisms including microorganisms that fall under RG 1
- Keeping and rearing of terrestrial arthropods that have been challenged or infected with GM organisms that fall under RG1

A GI-BSL2 arthropod facility is not appropriate for the following:

- Activity with any GM arthropod that require a higher BSL for containment than GI-BSL1
- Activity with GM organisms unless they are integral to the activity being conducted in the facility
- Housing/keeping/rearing of animals (other than terrestrial arthropods) or aquatic organisms (including amphibians)
- Growing of any plants other than those required as part of an activity with arthropods that are LMO

Arthropod Facility (GI-BSL1)

a) The insectarium should be located out of the flow of general traffic, avoiding hallways or placing arthropods in closets.

b) The area should be designed to allow detection of escaped arthropods. For example, walls of the facility should be painted white or with a contrasting colour to the arthropod that is kept in the facility.

c) Entrance to the arthropod facility should be posted with appropriate signage identifying the type of arthropod facility and make aware of the presence of arthropod vectors. The contact information of the laboratory supervisor or other responsible persons should be listed.

d) Door openings should be covered by rigid panels, glass, screens, plastic sheets or cloth to minimise escape and entry of arthropods.

e) Windows should be covered with mesh or screen to effectively prevent escape of the smallest arthropods contained within.

f) All air-conditioning inlets and outlets should be covered with suitable-sized mesh to prevent arthropods passing through.

g) The GI-BSL1 facility should be fitted with a suitable electric insect-control unit or an appropriate insecttrap.

h) Materials unrelated to arthropod rearing and experimentation (e.g. unused containers and clutter) that provide breeding sites and harborages should be minimised.

i) Arthropod facility should contain a sink for hand-washing.

Work practices (GI-BSL1)

a) The facility senior management is required to establish policies and procedures whereby access to the facility is restricted to authorised persons. Personnel providing support services in the facility are instructed on appropriate safeguards to ensure their safety. Access to the facility should be restricted by means of secure, locked doors.

b) An institutional biosafety manual describing arthropod facility practices and SOPis prepared and adopted for use in the insectary.

c) Eating, drinking, smoking and the storage of food for human use is not permitted in the arthropod facility.

d) Gloves, protective clothing (e.g. white laboratory coatsor gowns) and footwear are worn at all times in the insectary when handling blood and vertebrate animals and removed before leaving the arthropod facility. Personal protective equipment should be worn as appropriate (e.g. respirators for arthropod-associated allergies, particle masks, head cover).

e) All persons handling the GM arthropods or arthropods associated GM organisms should wash their hands after removing gloves and before leaving the facility.

f) All arthropods should be kept in suitable containers. All containers should be clearly labelled giving species, strain/origin, date of collection, responsible investigator and a central logbook of all GM arthropod stocks kept in the facility. Imported GM arthropod should be indicated as such in the logbook.

g) Arthropods and LMAs should be kept in separate containers except where genetic crosses have to be made. Such events should be clearly labelled and the arthropods contained therein should be treated as LMO.

h) Unintended introduction of arthropods from within the insectary should be prevented by cleaning work surfaces after a spill of materials, including soil or water that might contain viable eggs. Pools of water should be mopped up immediately.

i) Practices should be in place such that arthropods do not escape by in advertent disposal in primary containers. Cages and other culture containers should be appropriately cleaned to prevent arthropod survival and escape (e.g. heated to or chilled below the lethal temperature).

j) Cages used to hold arthropods effectively prevent escape of all stages. Screened mesh, if used, is durable and of a size appropriate to prevent escape. Non-breakable cages are recommended. Bags, rearing trays and so on effectively prevent leakage and escape.

k) All stages of arthropods should be rendered inviable before disposal. All wastes from the insectary (including dead arthropods and rearing medium) should be transported from the insectary in leak-proof, sealed containers for appropriate disposal in compliance with applicable institutional or local requirements (refer to section 13).

l) Living arthropods should not be taken out from the facility except when they are being transferred to another containment facility or to an approved release site. Arthropods taken into or out of the facility should be carried in covered containers (refer to Section 16).

m) Autoclaving or incineration of arthropods and their life stages infected with a non-pathogenis recommended. Alternatively, these organisms may be killed with hot water or by freezing before disposal.

n) Personnel should take appropriate precautions to prevent transport or dissemination of arthropods from the insectary on their persons or via the sewer.

o) A programme should be implemented to prevent in filtration of non target arthropods (e.g. houseflies, cockroaches, spiders) and rodents

to effectively preclude predation, contamination and possible inadvertent infection by methods appropriate to the organisms.

p) An effective arthropod management programme is recommended to monitor any unintended release of arthropods. Escaped arthropods should be recovered and rendered inviable before proper disposal.

q) Arthropods fed on host animals should be prevented from accidental transfer to host cages. When handling/removing animals after exposure to arthropods, precautions should be taken to prevent arthropod escape through screens, covers, and by flying. Host animals are inspected closely (e.g. concealment in fur, ears, crevices) and the primary container is sufficiently robust to prevent escape during feeding.

r) Policies for the safe handling of sharps should be instituted. Needles and syringes or other sharp instruments should be restricted in the arthropod facility for use only when there is no alternative. Syringes that re-sheath the needle, needle-less systems and other safe devices should be used when appropriate. Plastic ware should be substituted for glassware whenever possible.

s) The insectary supervisor should be notified promptly of any unintentional release of arthropod vectors. A system is established for the reporting of accidents, accidental releases, incidents, exposures and for the medical surveillance of potential laboratory associated illnesses.

Staff wearing personnel protective clothing: gloves, protective gowns, head cover, and shoe covers
Source: IMR

9.2 Biosafety Level 2 (GI-BSL2)

General

A facility classified as GI-BSL2 is appropriate for conducting activities listed below:

- Experiments with terrestrial arthropods that are modified with genes from other organisms including microorganisms that fall under RG 2
- Keeping and rearing of terrestrial arthropods that have been challenged or infected with GM organisms that fall under RG2

A GI-BSL2 arthropod facility is not appropriate for the following:

- Activity with any GM arthropod that require a higher BSL for containment than GI-BSL2
- Activity with GM organisms unless they are integral to the activity being conducted in the facility
- Housing/keeping/rearing of animals (other than terrestrial arthropods) or aquatic organisms (including amphibians)
- Growing of any plants other than those required as part of an activity with arthropods that are LMO

Arthropod Facility (GI-BSL2)

In addition to the facility design features specified for GI-BSL1, the following additional features are essential:

- The GI-BSL2 facility for arthropods should be fitted with a double self-closing door that provides a seal sufficient to contain the arthropod species under study. The door should preferably be unmeshed. The facility should also be free of other possible escape routes e.g. false ceilings. Windows should be permanently sealed. Sink outlets should have water traps or other suitable measures to prevent escape of arthropods.
- A dedicated hand basin of the hands-free operation type should be provided near the exit within the arthropod facility.
- The arthropod facility should be inspected regularly to ensure all containment features are intact.

Arthropod cages constitute the primary containment in insectariums. Adult mosquitoes kept in rearing cages. Access is via a 60cm long cloth sleeve (surgical stockinet) that fits an opening on 1 side of the cage. The cages are kept in ventilated secure cabinets. (Source: IMR)

Work Practices (GI-BSL2)

In addition to the work practices described in GI-BSL1 the following work practices should be observed:

- All doors to the arthropod facility should be locked for the duration of the work except for those periods when personnel are working inside it.
- Screens and filters especially at the air-conditioning inlet and outlets should be cleaned regularly (in accordance with manufacturer's specifications when provided).
- Work surfaces should be decontaminated after use and after any spill of viable material.
- All containers that contain or have contained GM arthropod should be decontaminated prior to disposal. The room should be kept free of material wastes.

9.3 Biosafety Level 3 (GI-BSL3)

General

A facility classified as GI-BSL3 is appropriate for conducting activities listed below:

- Experiments with terrestrial arthropods that are modified with genes from other organisms including microorganisms that fall under RG 3
- Keeping and rearing of terrestrial arthropods that have been challenged or infected with GM organisms that fall under RG3

A GI-BSL3 arthropod facility is not appropriate for the following:

- Activity with any GM arthropod that require a higher BSL for containment than GI-BSL3
- Activity with GM organisms unless they are integral to the activity being conducted in the facility
- Housing/keeping/rearing of animals (other than terrestrial arthropods) or aquatic organisms (including amphibians);
- Growing of any plants, other than those required as part of an activity with arthropods that are LMO

Arthropod Facility (GI-BSL3)

The design, construction and major changes to the GA-BSL3 facility should be certified by a competent authority/organisation approved by the NBB before commencement of work.

In addition to the facility design features specified for GI-BSL1 and GI-BSL2, the following additional features are essential:

a) A hazard warning sign incorporating the universal biohazard symbol and the level of containment together with access restrictions should be displayed on the access door to the laboratory work area. The hazard warning sign may also identify the agent, name and telephone number of the Laboratory Supervisor or other responsible persons.

b) GI-BSL3 door signs will be posted on access doors.

c) The arthropod facility should be provided with an access room. The access room should be fitted with insect-control units for example an electric insect-control device or an ultra-violet insect zapper. Access room doors should be sealed to be arthropod-proof. If risk assessment requires additional mitigation measures for arthropod containment, an anteroom may be provided with a sink and vacuum system to enable personnel to remove any arthropods, eggs or larvae from their person before leaving the facility.

d) The joints between any structural components of the arthropod facility should be sealed. Transparent sections (e.g. windows) should be made of an impact-resistant material. Windows should be permanently sealed.

e) Air supply and exhaust ducts should be fitted with fine screens of suitable size and designed to preclude arthropod escape.

f) The arthropod facility should be provided with contained cabinets for handling or transfer of arthropods.

g) The GI-BSL3 facility should be tested and certified annually at least every 12 months.

Work Practices (GI-BSL3)

In addition to the work practices described in GI-BSL1 and GI-BSL2, the following work practices should be observed:

a) Personnel providing support services in the facility should be accompanied by authorised persons.

b) Personnel should wash their hands on entering and leaving the arthropod facility. When entering, personnel should put on shoe covers, laboratory overalls and hair covering in the anteroom. Before personnel leave the arthropod facility, any stray arthropods should be removed from these garments with the vacuum device provided, and the garments removed and kept in the anteroom between uses. Prior to washing (or disposal), garments should be sealed in bags and autoclaved.

c) Materials taken into and out of the arthropod facility should be suitably treated for destroying or removing all stages of the life-cycle of arthropods and their pathogens. This requirement applies to soil substitutes and soil. Soil substitutes which can be readily decontaminated should be used in preference to soil.

GENETIC MODIFICATION OFAQUATIC ORGANISMS (GF-BSL)

CHAPTER 10

The following guidelines specify physical containment and work practices suitable to conduct experiments with GM aquatic organisms and aquatic organisms associated with GM organisms. The main objective of aquatic organism containment is to ensure that GM aquatic organism or the aquatic organism associated GM organisms are securely contained and safely handled and to prevent unintentional release of these GM aquatic organisms or associated GM organisms into natural waterways.

In live aquatic animal holding facilities, water is the transport mechanism and the tanks represent the primary containment devices. Tanks must be contained to reduce the risk of spillage. In these facilities, potentially contaminated water is discharged from flow-through and recirculation-based aquatic animal holding systems. Other sources of waste water include:

- Wash water discharge collected in the floor drains
- Waste water from tank and boot cleaning
- Spillage from equipment (nets, transport tanks and pails, plumbing equipment)
- Regularly scheduled maintenance of animal holding units (flushing/ removal of sludge from drains and pipes, debris from animal holding units)
- Experimental procedures

In addition, spread or movement of GM aquatic organism or aquatic organism associated GM organism can also occur via fomites such as clothing, boots, hands, netting material, transport containers and equipment.

Work with aquatic organisms presents a variety of special hazards including exposure to physical hazards and chemical hazards. In addition, allergic conditions may result from handling aquatic organisms and their tissues or chemicals used in the facilities. Personnel should be familiar with and have access to Material Safety Data Sheets (MSDS) for all chemicals used. Employees should be aware of potential allergies that could be aggravated by working with live aquatic organisms or their tissues. Although few zoonotic infection transmitted by aquatic organisms are recognised, care must be taken when working with any GM aquatic organism or aquatic organism associated LMO, particularly those that have not been studied extensively.

In addition to the requirements and recommendations set out in this section, the design of the aquatic LMO facility should adhere to Malaysian guidelines such as *Principles and Guide to Ethical Use of Laboratory Animals, Ministry of Health, 2000* to meet the physiological, husbandry and welfare requirements for the animal species under investigation.

The following guidelines for aquatic organism facility and operating procedures are regarded as a minimum requirement for genetic modification work with aquatic organisms which fall under GF-BSL1 and GF-BSL2. Those planning to be involved in GF-BSL3 will be considered on an individual basis following a risk assessment done by IBC. Investigators submitting proposals for research involving GM aquatic organisms should describe the containment facilities to be used and procedures proposed for treatment of waste water from the facility.

10.1 Biosafety Level 1(GF-BSL1)

General

A facility classified as GF-BSL1 is appropriate for conducting activities listed below:

- Experiments with aquatic organisms that are modified with genes from other organisms including microorganisms that fall under RG 1
- Experiments with aquatic organisms that are modified with genes originating from the same family
- Keeping or rearing of aquatic organisms, including amphibians and aquatic plants associated with GM organisms that fall under RG 1

A GF-BSL1 Aquatic Organism Facility is not appropriate for the following:

- Activity with any arthropods that are LMO that require a higher BSL for containment than GI-BSL1
- Activity with GM non-aquatic microorganisms, unless they are integral to the activity being conducted in the facility

- The housing/keeping/rearing of terrestrial animals or terrestrial arthropods
- The growing of any terrestrial plants

Aquatic Facility (GF-BSL1)

a) The rearing area should be confined in a secured building and work on GM aquatic organisms should not be performed in natural waterways.

b) Appropriate signage should be posted at entrances identifying the type of aquatic facility. The contact information of the laboratory supervisor or other responsible persons should be listed.

c) All effluent water should leave the facility through a common drain and should be passed through at least two screens. Appropriately sized screens or filters may be used to collect some of the sediment and organic material before it enters the liquid effluent treatment system and should be able to retain the smallest life history stage of the organisms in use. The screens should be cleared regularly to prevent blockage and overflow.

d) Effluent water should not be discharged into a major system containing related species.

e) The building should be structurally sound and of sufficient elevation to preclude flooding or unintentional escape of these transgenic organisms.

f) To prevent escape of any GM aquatic organisms into the sewerage system, an appropriate sized screen should be fitted to outlets used for disposal of tank water to prevent the passage of smallest life history stage of the organisms.

g) Aquatic facility should contain a sink for hand-washing.

Work Practices (GF-BSL1)

a) Access to the aquatic facility and support areas should be limited to authorised personnel only. Doors to the facility should be locked for the duration of the experiment except for those periods when personnel are actually working inside it (this does not apply to an open area within a laboratory).

b) A biosafety manual that covers basic safety and general facility operations relating to biosafety protocols should be available to all personnel in the facility.

c) SOP describing the entire chain of events from receipt of aquatic LMO material to decontamination and disposal should be prepared

and personnel should be trained and follow the SOP. Procedures should also include prevention of release of contaminated materials into drainage systems unless linked to a decontamination system.

d) Aquatic organism that are LMO and those that are non LMO should be kept in separate containers and should be clearly labelled. All aquatic organism contained therein should be treated as LMO.

e) An Emergency Response Plan (ERP) should be available that describes emergency procedures in the event of accidents, fires, spills, power loss and other situations. Plans should cover emergency egress procedures, corrective actions and notification of key personnel.

f) Eating, drinking, smoking and the storage of food for human use is not permitted in the arthropod facility.

g) Appropriate protective clothing, properly fastened, should be worn by all personnel, as well as by visitors, trainees and others when working in the facility. Laboratory clothing should not be worn in non-laboratory areas. Laboratory clothing must be stored separately from street clothing. Gloves should be worn to avoid inadvertent contamination of samples and work areas. Gloves are to be removed when leaving the facility and decontaminated prior to disposal. Only completely enclosed (toes and heels) footwear (e.g. boots) should be worn in the facility.

h) Hands should be washed after removing gloves and before leaving the facility.

i) All handling procedures must be designed and carried out to minimise the creation of aerosols.

j) All organisms and contaminated waste should be rendered non-viable prior to disposal. Tank water used for work with LMO or tank water that has any potential to contain embryos, sperm, eggs, larvae or other life stage of infected aquatic organisms, should be treated to ensure inactivation of viable material before disposal or cleaning for reuse.

k) Since many aquatic species have sticky eggs which attach firmly to substrates, nets and other equipment used in the tank during spawning should be sterilised after use. The tanks should be decontaminated after use by a procedure of demonstrable efficacy for the species used.

l) Contaminated work surfaces should be decontaminated with an appropriate disinfectant.

m) Leak-proof containers should be used for the transport of GM aquatic

organism materials within facilities (e.g. between laboratories in the same facility). Infectious materials to be transported from the facility should be done in accordance and compliance with the appropriate regulatory authority (e.g. *Transportation of Dangerous Goods Regulations*).

n) Traffic flow patterns from clean to dirty areas should be established and adhered to (e.g. movement from least to most contaminated areas).

o) The use of needles, syringes and other sharp objects should be limited to where necessary.

p) All spills, accidents, overt or potential exposures to infectious materials should be reported immediately to the facility supervisor, written records of such incidents must be kept.

q) An effective rodent and insect control programme must be maintained.

10.2 Biosafety Level 2 (GF-BSL2)

General

A facility classified as GF-BSL2 is appropriate for conducting activities listed below:

- Experiments with aquatic organisms that are modified with genes from other organisms including microorganisms that fall under RG 2
- Keeping or rearing of aquatic organisms including amphibians and aquatic plants associated with GM organisms that falls under RG 2

A GF-BSL2 Aquatic Facility is not appropriate for the following:

- Activity with GM non-aquatic microorganisms unless they are integral to the activity being conducted in the facility
- Housing/keeping/rearing of terrestrial animals or terrestrial arthropods
- Growing of any terrestrial plants.

Aquatic Facility (GF-BSL2)

In addition to the facility design features specified for GF-BSL1, the following additional features are essential:

a) The site selection process for a containment facility should include an assessment of local aquatic programmes as well as the local environment. The risks to aquaculture and the environment especially susceptible aquatic species, including the impact of

possible GM aquatic organism releases should be considered before any work is begun with a particular aquatic animal pathogen. In areas prone to natural disasters, buildings and support systems for containment facilities should meet more stringent building code requirements.

b) Appropriate signage indicating the nature of the aquatic animal pathogens being used (e.g. type and containment level) should be posted on the entry door to each laboratory. If there are special provisions for entry, the relevant information should be included on the sign.

c) Containment facilities require frequent wash downs of surfaces and all facility surfaces such as walls and floors should be resistant to chemical attack, absorption and the effects of salt water in some cases. Bench-top surfaces or other non-absorbent solid surfaces should have epoxy finishing.

d) To facilitate decontamination and maintenance, systems such as liquid effluent treatment systems and HEPA filter housings should be located as close to the containment perimeter as possible and consideration must be given to installing valves to isolate sections of plumbing and ductwork.

e) Liquid effluent treatment systems must be designed with convenient sampling ports allowing for the collection of samples of treated effluent to monitor decontamination efficacy.

f) The provision of dedicated equipment, storage areas and paperwork workstations inside the containment zone should be considered to minimise traffic into and out of the containment facility.

g) Storage space is necessary for supporting operations, cleaning equipment, spill management, emergency safety response tools and equipment.

h) Air handling systems should be designed to accommodate the additional moisture that is generated in live aquatic animal holding facilities and that auxiliary localised dehumidifiers may be required.

i) Inward directional airflow is recommended.

j) A dedicated hand basin of the hands-free operation type should be provided near the exit within the animal facility.

k) Circuit breakers and shut-off valves should be located outside the containment perimeter to facilitate maintenance.

l) A delivery systems must be taken into consideration to ensure proper containment in the aquatic LMO holding facilities and disinfection of the transport mechanism (e.g. container or vehicle) used.

Work Practices (GF-BSL2)

In addition to the work practices described in GF-BSL1, the following work practices should be observed:

a) Visitors and any untrained personnel should be accompanied by authorised personnel in order to work in the aquatic facility.

b) All personnel entering GF-BSL2 should sign a log book and indicate the date and time of each entry and exit.

c) Personnel should receive training on the potential hazards associated with the work involved and the precautions required to prevent exposure to infectious substances and potential zoonotic agents. Training records should be signed by both employee and supervisor. All persons entering the containment zone should receive training in the operational procedures for entry and exit. Trainees should be accompanied by a trained staff member.

d) Persons entering the facility should have access to and wear appropriate dedicated protective equipment such as respirator, particle mask and eye protection when required.

e) Protective clothing and equipment should be removed before exiting the facility and the contaminated clothing should be autoclaved prior to laundering (unless laundering facilities are located within the facility and have been proven to be effective in decontamination). Some activities or projects may require more thorough entry and exit procedures.

f) Autoclaves and other decontamination processes are to be verified to ensure appropriate operation and validated using representative loads with appropriate biological indicators.

g) Aquatic organism carcasses and tissues must be incinerated or processed using technology proven to effectively decontaminate all tissues. Where such materials should be transported for decontamination outside the facility, this should be done using leak-proof and impact resistant containers labelled appropriately.

h) Periodic inspections of the facility should be made to check for inward directional airflow (if applicable), faults and deterioration (e.g. deteriorated door seals). Corrective action should be taken and records maintained.

i) Primary containment devices (e.g. biological safety cabinet) should be used for procedures that may produce aerosols and that involve high concentrations or large volumes of aquatic organism pathogens. Aeration should be slowed or stopped before the removal of covers to prevent the aerosolisation of pathogens.

CHAPTER 11

BIOLOGICAL SAFETY CABINETS

Biological safety cabinets are classified as Class I, Class II or Class III cabinets.

a) A Class I cabinet is a ventilated cabinet for personnel protection. Air flows inward, away from the operator. The exhaust air from this cabinet filters through a HEPA filter. This cabinet is used in three operational models:

- with full-width open front
- with an installed front-closures panel (having four 12-cm diameter openings) without gloves
- with an installed front-closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 30 m per minute or greater.

b) A Class II cabinet is a ventilated cabinet for personnel and product protection. It has an open front with inward airflow for personnel protection and HEPA filtered mass airflow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 30 m per minute or greater.

c) A Class III cabinet is a closed-front ventilated cabinet of gas-tight construction, which provides the highest level of personnel protection among biological safety cabinets. The interior of the cabinet is protected from contaminants outside of the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 1.25 cm water gauge. All air supply is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters before being discharged to the outside environment.

Biological Safety Cabinet

Biological safety cabinets are required to pass an air barrier containment test. The test is the direct determination of the effectiveness of containment by the air barrier and is part of the certification required by the biological safety cabinet annually. All cabinets should be checked for containment efficiency and safety before initial use, after any modification including change of HEPA filter, after relocation and on an annual basis.

The cabinets should be decontaminated with formal dehyde gas or an equivalent disinfectant before testing when there have been used for handling RG 2, 3 or 4 microorganisms. Penetration of the decontaminants throughout all the sections of the cabinets is essential. The biological safety cabinet should be tested and certified annually at least every 12 months.

CHAPTER

12 TREATMENT METHOD FOR BIOHAZARDOUS WASTE

12.1 Disposal of Treated Biohazardous Waste

Table 1: Summary of Treatment, Disposal and Containment of Biohazardous Waste

Category of Waste	Container	Treatment	Disposal	Notes
MICROORGANISMS				*For BSL3 and BSL4, autoclaved waste is disposed via H or J or specific procedures as found in the text. For clinical waste, all waste has to be disposed via H or J.
• all microbial cultures	A	D E F G	H*	
• potentially infected waste	A	D E F G	H*	
• liquid infected waste	A	D E F G	H*	
• handling materials such as gloves, paper and disposable plastics	A	D E		
PLANT				Autoclave prior to disposal into yellow bag *For BSL3 and BSL4, autoclaved waste are disposed via H or J.
• plants and seed	A B	D E	X*	
• other plant parts/ tissues	A B	D E	X*	
• growth media/ soil substitute	A B	D E	X I	
• handling materials such as gloves, paper and disposable plastics	A	D E	X*	
ANIMAL				Keep separate from all other waste Large carcasses for incineration
• animal carcasses	A B	D E	H J	
• tissue and body parts	A B	D E	H J	
• whole blood, serum, plasma, and other blood components / fluids	A B	D E G	I	
• bedding from animal cages	A B	D E	H J	
• handling materials such as gloves, paper and disposable plastics	A B		H J	

INSECTS & ATHROPODS				Shall be treated to ensure inactivation of viable material before disposal
• whole and animal parts	A B	D E	H J	
• various stages of life cycle	A B	D E	H J	
• culture medium	A B	D E	H J	
• nets, grill or mesh, gauze, cotton	A B	D E	H J	
• handling materials such as gloves, paper and disposable plastics	A	D E	H J	
AQUATIC ORGANISMS				Tank water should be treated to ensure inactivation of viable material before disposal
• whole and animal parts	A B	D E	H J	
• nets, grill or mesh fitted to the outlets of the tank	A B	D E	H J	
• embryos, sperm, egg or larvae	B	G	I	
• used tank water	B	G	I	
• handling materials such as gloves, paper and disposable plastics	A B	D E	H J	
SHARPS				Keep separate from all other waste
• discarded syringes, needles, scalpels and blades used in non-clinical activities	C		J	
• other sharp objects	C		J	
• disposable and broken glassware	C		J	

The Legend of Table 1is detailed below:

1. CONTAINER REQUIREMENTS FOR BIOHAZARDOUS WASTE

A. Heavy duty autoclavable plastic bag with biohazard symbol (Blue / Clear)

B. Heavy duty leak proof container with biohazard symbol

C. Puncture resistant container (Sharps container)

2. TREATMENT OF BIOHAZARDOUS WASTE

D. Autoclave [121°C,15 psi, 30 mins (minimum)]. Time required depends on the amount of waste (autoclave load), the presence of water and the type of container used

E. Incinerate

F. Dry heat [160°C, 2 hr (minimum)]. Time of exposure begins after attaining the specific temperature and does not include lag time

G. Chemical disinfection (5-10%) hypochlorite or IBCa pproved chemical disinfectant or sterilant used according to manufacturer's instruction

3. DISPOSAL OF TREATED BIOHAZARDOUS WASTE

X. The autoclave bag is placed into a garbage bag and disposed as normal waste by the service provider

H. The autoclaved bag is placed into a biohazard container for removal by service provider for incineration

I. Disinfected liquid is flushed into the sewer system

J. Collection and disposal for incineration by service provider

12.2 Labelling Requirements

Containers of treated biohazardous waste should be labelled to indicate the method of treatment.

12.3 Written Procedures and Records

Each facility is required to maintain written records which at a minimum to contain the following information:

- Date of treatment
- Method/conditions of treatment
- Name of the persons performing the treatment
- A written procedure for the operation and testing of any equipment used and a written procedure for the preparation of any chemicals used in treatment
- Efficacy of the processes used for waste treatment should be verified by showing records to proof compliance to specified performance standards

DISPOSAL OF LMO AND RELATED WASTES

CHAPTER 13

It is the clear responsibility of all research workers to ensure the safe and correct disposal of all wastes produced in the course of their work. Improper and irresponsible disposal of biological wastes down drains or into the atmosphere is forbidden by law [Environmental Quality Act 1974, Environmental Quality (Scheduled Wastes) Regulations 1989; Biosafety Act 2007]. Proper segregation of laboratory waste is essential for a safe work environment.

The following guidelines are intended to provide information and ensure compliance with local and international regulations and recommended procedures for contained use of LMO.

13.1 Responsibility

The Laboratory Supervisor or other person with operational responsibility should ensure compliance with requirements mentioned in the following subsections.

13.2 Biological Waste Segregation and Handling

Biological waste should be segregated from other types of waste at the point of origin into the following categories:

a) Infectious and Potentially Infectious Waste

   i) All biohazardous materials and all contaminated equipment or apparatus should be sterilised before being washed, stored or discarded. Autoclaving is the preferred method. Each individual working with biohazardous material should be responsible for its sterilisation before disposal.

      Note: Dry hypochlorites or any other strong oxidizing material, should not be autoclaved with organic materials such as paper, cloth, or oil: OXIDIZER + ORGANIC MATERIAL + HEAT = MAY PRODUCE AN EXPLOSION

ii) Sterilisation by autoclaving. It is advisable to review the type of materials being handled and to establish standard conditions for sterilisation. Treatment conditions to achieve sterility will vary in relation to the volume of material treated, its contamination level, the moisture content and other factors. General criteria for sterilisation of typical materials are presented below:

* Laundry: 121°C for a minimum of 30 minutes.
* Biohazard waste: 121°C for at least 30 minutes per bag. Sizes of the autoclave and of the bags greatly affect sterilisation time. Large bags in a small autoclave may require between 30 minutes and 1 hour.
* Glassware: 121°C for a minimum of 25 minutes.
* Liquids: 121°C for 25 minutes for each gallon.
* Animals & bedding: Steam autoclaving not recommended (sterilisation time required would be at least 8 hours). Incineration in an approved facility is the recommended treatment of these wastes.

b) Other Biological Waste

i) For disposal purposes, all GM material should be treated in the same way as infectious and potentially infectious material. All GM wastes should be inactivated prior to leaving the facility. The preferred method is autoclaving, although incineration or chemical disinfection (e.g. treatment with household bleach) may be appropriate or necessary in some cases).

ii) Storage of all non-inactivated waste in this category is restricted to within the generating laboratory. GM waste should be held in a closed/covered biowaste container and preferably should not be stored longer than 24 hours prior to inactivation.

iii) Biological waste containers and bags for GM materials should be labelled with the biohazard symbol.

iv) Filled or partially filled biological waste containers and boxes should not be held for more than 14 days.

v) Stock solutions of suitable disinfectants should be maintained in each laboratory for disinfection purposes.

c) Sharps Waste

Sharps are items that are capable of puncturing, cutting, or abrading the skin, e.g. broken plastic or broken glassware, glass or plastic

pipettes, scalpels, razor blades, needles, hypodermic needles. These instruments are used to cut or penetrate living GM tissues. Sharps should be placed in designated hard puncture resistant leak-proof plastic sharps containers.

The sharps container should be closed when it is ¾ full and not stored for more than 30 days before disposal by the appointed service provider.

d) Contaminated Disposable Glassware

All contaminated glassware should be sterilised by autoclaving or with suitable disinfectant before disposal. If disinfection is preferred, the glassware should be placed into a suitable receptacle containing an appropriate disinfectant. After a minimum of 12 hours soaking, the container is drained and the glass disposed of in a sharps container. Appropriate PPE should be worn for this procedure e.g. robust gloves, eye protection and a laboratory coat.

e) Contaminated Re-useable Glassware

i) Graduated pipettes

These are placed in the marked plastic containers so that the pipettes are completely submerged in an appropriate disinfectant (chlorine-based) for a minimum of 12 hours, then drained, rinsed and autoclaved for re-use.

ii) Glassware and reusable plasticware

These are collected in labelled, lidded containers and are either autoclaved or disinfected. After autoclaving or disinfection of any GM contents, they are disposed of in the biohazard bags and the glassware or plasticware is processed for re-use.

It may not be appropriate to autoclave some glassware or plasticware, usually because of fragility or size. In this situation all contaminating solid or liquid should be removed into an appropriate container and disinfected or autoclaved. The glassware or plasticware should then be disinfected with a chemical appropriate to the contaminant and the equipment.

f) Disposable Plastic Ware and Infected Solids

i) General

This material is placed in the biohazard labelled autoclavable bags. The bags should not be over-filled and should fit into the autoclave crates.

ii) Plant work

All waste plant tissue, soil, soil substitutes and the containers should be placed in an appropriate container and autoclaved or sent for incineration.

g) Contaminated Liquids

Small amounts of liquid remaining in reusable glassware should be autoclaved before washing and reuse. Pathogenic material should be autoclaved prior to disposal. As for contaminated liquid effluent from aquatic facility, mechanical filtration of water that called de-bulking, is useful as a first stage of liquid effluent treatment. However, a secondary treatment such as chemical, heat, gas, ozonation, irradiation, UV or other method of treatment is also necessary to ensure effective decontamination. Given the large volumes of water involved, decontamination of waste water requires the appropriate contact time to ensure effective inactivation of infectious agents.

h) Pipette Tips (or other Disposable PlasticWare)

This material should be placed into the approved containers (e.g. beakers lined with small autoclave bag or similar containers are also acceptable). When these are full, they are placed in plastic autoclave bags for autoclaving.

i) Chemically Contaminated Items

Chemically contaminated items (e.g. DNA extraction tubes contaminated with phenol/chloroform, specimen cups containing formalin, chemically contaminated gloves) should be handled as chemical waste.

NON-INFECTED WASTE

CHAPTER 14

Conventional waste which does not fall under the category of any infectious waste, sharp, chemical, radioactive, for example paper or food refuse.

This will be placed into ordinary waste bins (these bins should not be yellow) that will be emptied by the cleaners in all rooms, except laboratories. In laboratories, cleaners may be allowed to empty the bins for non-laboratory material e.g. card board boxes, paper only under supervision.

CHAPTER 15

BIOLOGICAL WASTE PACKING, LABELLING & TRANSPORT

a) Biowaste Boxes

- Sturdy cardboard biowaste boxes displaying the biohazard sign are used as the terminal receptacle. The boxes are lined with a biohazard bag.
- Boxes should not be over-filled.
- All seams should be taped and labelled with date, Laboratory Supervisor's name and telephone number.
- Biowaste boxes should not be kept for more than 30 days.

b) Biohazard Bags

- Liquids should not be put into the bags. The bags should be labelled with date, Laboratory Supervisor's name and telephone number.
- Biohazard bags are placed in a biowaste box for disposal.
- The laboratory should order/supply these bags

c) Sharps Containers

- The bags should be labelled with date, Laboratory Supervisor's name and telephone number.
- Close when three quarters full and inform service provider for disposal.
- The laboratory should order/supply these sharps containers.

d) Transport

- Biohazardous waste is transported outside of the laboratory (e.g. to an autoclave or incinerator) in a closed, leak-proof bag or container. Bags should be placed in a leak-proof tray.
- Non-inactivated waste should not be left unattended.
- Biowaste boxes designated for pick-up by the service provider should be stored in a secure area and should be sheltered from inappropriate weather conditions.
- All laboratory bins should have a biohazard sign and should be lined with a standard biohazard plastic bag. All material placed in these biohazard bins should be incinerated. This system is approved by the service provider.
- Pathogenic organisms and all infectious waste should be autoclaved before being placed in the biohazard bins. The bin should not be overfilled.
- As all GM material should be double-contained when transported outside the facility, the bin should be brought to the laboratory so that the full, sealed bags can be placed in them and then returned to the designated location.

CHAPTER 16

BIOLOGICAL WASTE PACKING, LABELLING & TRANSPORT

For the movement and transport of LMO and related materials (including import and export), the following shall apply:

- The regulatory authorities (IBC and NBB) shall be notified using the relevant forms.
- LMO being transferred should be packaged in secure containers capable of preventing material loss during transportation. LMO should be kept separate from other materials.

Specific transfer trolley used in CSIRO to transfer LMO and related materials

General procedures for the packaging and transport of LMO are tabulated below:

Microorganism and cell lines	• Cultures of microorganism or cell lines should be placed in a primary container that is secure closed-watertight and sift-proof • The primary container should be placed within a sealed and leak-proof secondary container, which is resistant to breakage or water damage. • Padding material should be placed at the top, bottom and sides between primary and secondary containers to prevent breakage of the primary container during transport. • Sufficient absorbent material should be included (e.g. paper towel) to absorb the entire contents of the primary containers in case of breakage or leakage. • For local surface transport, each set of primary and secondary containers should then be enclosed in an outer container made of corrugated fiberboard, corrugated cardboard, wood or other material of equivalent strength. For air transport and shipment, the primary, secondary and outer container should follow International Air Transport Association (IATA) requirements.
Plant	**Seeds, propagules or plant parts** • These materials should be transported in a durable bag or a sealed envelope or package constructed of tear and moisture resistant material as primary container. • Primary container can contain only plant material of a single type derived from one line of a single event. • The primary container should be placed within a sealed, leak-proof secondary container which is resistant to breakage or water damage. **Seedlings and plants** • Seedlings and small plants should be transferred in a non-breakable container as primary container. • The primary container should be placed within a leak-proof secondary container, which is resistant to breakage or water damage. • Sufficient packing material should be included around the primary container to prevent movement and damage in the transport. • GM plants that are two meters or more in height, and have not started flowering, will need to be transported in a fully covered vehicle which has a floor layered with a durable plastic. No plants bearing flowers or buds should be transported. The vehicle compartment containing the GM plants should be sprayed with pesticide before and after transportation. • All plant materials for shipment to a foreign country should adhere to the Biosafety Act 2007 and also plant quarantine regulations of Malaysian Quarantine and Inspection Services (MAQIS) and of the recipient country.

Animal	• Animals should be placed in primary shipping containers made of a sturdy, crush-proof frame of wood, metal or material of equivalent strength, surrounded by escape-proof mesh or netting of a strength and mesh size sufficient to prevent the escape of the smallest organisms in the shipment, with edges and seams of the mesh or netting sealed to prevent escape of organisms. • Each primary shipping container should be securely placed within a larger secondary shipping container made of wood, metal or equivalent strength material. • The primary and secondary shipping containers should then be placed securely within an outer shipping container made of corrugated cardboard, wood or other material of equivalent strength. • The outer container may have air holes or spaces in the sides or ends of the container, provided that the outer shipping container retains sufficient strength to prevent crushing of the primary and secondary shipping containers. • Animals should be treated in the most humane manner. Handling and treatment of animals during transport should follow requirements of Malaysian Guidelines on the Principles and Guide to Ethical use of Laboratory Animals (2000) and Guidelines for Humane Transportation of Research Animals (2006). • Animals for shipment to a foreign country should adhere to the Biosafety Act 2007 and also animal quarantine regulations of Malaysian Quarantine and Inspection Services (MAQIS) and the recipient country.
Arthropod	• Arthropods (any life stage) should be placed in a sealed escape proof primary container to prevent escape. • The primary container should be placed in a sealed, secure and leak-proof secondary container, which is resistant to breakage or water damage. • Sufficient packing material should be included around the primary container to prevent movement and damage in the transport. • For local surface transport, each set of primary and secondary containers should then be enclosed in an outer container made of corrugated fiberboard, corrugated cardboard, wood or other material of equivalent strength. For air transport and shipment, the primary, secondary and outer container should follow IATA requirements.

Aquatic Organism	• Aquatic organisms should be placed in a secure closed-watertight container which is leak-proof and sift-proof as primary container. • The primary container should be placed within a sealed and leak-proof secondary container, which is resistant to breakage or water damage. • A single primary container should not contain more that 1,000 ml of liquid. • Two or more primary containers, with combined volumes, should not exceed 1,000 ml when placed in a single secondary container. • Padding material should be placed at the top, bottom and sides between primary and secondary containers to prevent breakage of the primary container during transport. • Sufficient absorbent material should be included (e.g. paper towel) to absorb the entire content of the primary containers in case of breakage or leakage. • Each set of primary and secondary containers should then be enclosed in an outer shipping container made of corrugated fiberboard, corrugated cardboard, wood or other material of equivalent strength.

Additional Procedures

a) Labelling Requirements

Clearly label the outside of the package. The label should include:

- Contains LMO
- Biosafety level of containment
- Type of materials
- Amount of materials
- Contact details of person to contact in the event of an unintentional release

b) All containers used should be sanitised prior to filling and after the LMO have been removed, if intended to be re-used. Alternatively, containers should be destroyed after use by autoclaving or burning. Any residual materials recovered during the process of sanitisation should be rendered non-viable.

c) If an unintentional release of LMO during transport occurs, all attempts should be made to recover as much of the materials as possible. The location should be marked and treated in a manner that ensures that no additional release of materials occurs. Any corrective actions taken should be documented and the regulatory authorities notified.

d) After a corrective action is taken to address a compliance infraction, the authorised party should undertake a timely review of the situation to identify its cause(s) and then institute any changes in management practices or additional training of personnel to ensure that the situation is not repeated.

e) Adequate records of the transport of LMO as they move between research facilities, storage facilities and field trial sites should be maintained by IBC to ensure an adequate system is in place for tracking the movement of this material.

f) The shipper should notify the recipient of the date, kind and amount of material that will be sent before shipped. Upon receiving the material, the recipient should confirm that the shipment has arrived intact and that no material has been lost.

STORAGE OF LMO AND RELATED MATERIALS

CHAPTER 17

a) LMO should be stored at the destination facility in such a way that there is no release of LMO and related materials into the environment so as to avoid mixing with other LMO or conventional materials (e.g. filing cabinet, refrigerator, office, closet, cold room).

b) Where a storage area is used to store multiple samples of LMO, each item should be stored separately in a sealed, labelled container such as a primary container for shipment. LMO in storage should be clearly labelled to prevent mixing of materials.

c) Appropriate pest control should be implemented to ensure that pests do not damage storage containers, mix or remove LMO and related material from the storage facility.

d) Storage areas should be cleaned prior to and immediately following the period of storage. Any residue or other material recovered during cleaning or any material removed from storage for disposal, should be rendered non-viable.

e) All storage areas should be clearly labelled at the point of access as containing LMO and access should be limited to authorised personnel only. All personnel who have access to the storage areas should be adequately trained on the labelling, storage and disposal procedures.

f) Access to the area for the purpose of inspection should be provided to regulatory officials upon request, provided they present official identification documents and the inspection is undertaken at a reasonable time.

g) In the event of any suspected unintentional release of LMO from storage, the same procedures should be followed as previously described.

h) An inventory of all LMO in storage should be maintained. Sub-samples that may be removed from storage when required for experimental or other purposes should be recorded in the inventory list.

i) The storage area should be checked and maintained at regular intervals to avoid unintentional release of LMO into the environment and such inspections should be recorded. These inspections should include checks on the integrity of material packaging that may have been deployed.

CHAPTER 18

TRAINING

a) Employees who handle biological waste should receive training on the proper segregation, handling, packaging, labelling, storage, treatment and disposal of biological waste. Refresher training is arranged according to requirements.

b) Training may be accomplished through the IBC, informally in the laboratory setting or through formal training programmes. Records of the training sessions should be maintained for each employee, along with an outline of the training programme. Training records should be retained.

c) Besides formal training (refer to Guidelines for Institutional Biosafety Committees: Use of Living Modified Organisms and Related Materials, Section 5), Biosafety Officers, Principal Investigators, Laboratory Supervisors and Laboratory Workers should adhere strictly to all procedures. Refresher courses may be appropriately conducted either formally or informally.

APPENDIX 1
National Legislations and Relevant Documents

Relevant documents, including those mentioned in this Guideline are listed below.

1. Relevant Legislation

1.1 Biosafety Act 2007 and Biosafety (Approval & Notification) Regulations 2010

1.2 Environmental Quality Act 1974

1.3 Environmental Quality (Scheduled Wastes) Regulations 1989

1.4 Plant Quarantine Act 1976

1.5 Plant Quarantine Regulations of the Malaysian Quarantine and Inspection Services (MAQIS)

1.6 National Forestry Act 1984

1.7 Fisheries Act 1985

1.8 Occupational Safety & Health Act 1994

1.9 Occupational Safety & Health (Notification of Accidents Dangerous Occurrences, Occupational Poisoning & Occupational Diseases) Regulations 2004

1.10 Prevention & Control of Infectious Diseases Act 1988

1.11 Prevention & Control of Infectious Diseases (Importation & Exportation of Human Remains, Human Tissues and Pathogenic Organisms & Substances) Regulations 2006

1.12 Pathology Laboratory Act 2007

1.13 The Animal Ordinance 1953

1.14 Animal Rules 1962

1.15 Animal Importation Order 1962

2. Relevant Local Guidelines

2.1 Guidelines for Institutional Biosafety Committees: Use of Living Modified Organisms and Related Materials

2.2 Principles and Guide to Ethical Use of Laboratory Animals, MOH, 2000

3. Relevant Biosafety Guidelines and References

3.1 The International Air Transportation Association (IATA), *Dangerous Goods Regulations* (IATA online store – http://www.iata.org/)

3.2 Laboratory Biosafety Manual, 3rd Edition, World Health Organisation, 2004

3.3 Biosafety in Microbiological and Biomedical Laboratories 5th Edition, U.S. Department of Health and Human Services Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health, 2007

3.4 WHO Biorisk Management: Laboratory Biosecurity Guidance (WHO, 2006)

4. Other Relevant Guidelines

4.1 Guidelines for Humane Transportation of Research Animals (2006). Committee on Guidelines for the Humane Transportation of Laboratory Animals, National Research Council, The National Academies Press, Washington D.C. www.nap.edu

4.2 Containment Standards for Facilities Handling Aquatic Animal Pathogens First Edition; Canadian Food Inspection Agency (CFIA) www.inspection.gc.ca

APPENDIX 2
Risk Group (RG), Biosafety Level (BSL) and Pathogenicity Features of Features of Living Modified Microorganisms

Risk Group (RG)	Pathogenicity Features
RG1 low individual and community risk (BSL1)	A microorganism that is unlikely to cause human disease or animal disease of veterinary importance.
RG2 moderate individual risk, limited community, livestock or environment risk (BSL2)	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 exposure may cause serious infection. Infectious risk is via direct contact, ingestion or inhalation. Effective treatment, preventive and control measures are readily available and can be implemented to control disease transmission. Risk of spread to a community is limited.
RG3 high individual, low community risk (BSL3)	Organism, which may be an exotic or indigenous agent with potential to transmit disease mainly via aerosols. Disease caused is severe and may result in death. It could present a risk if spread in the community however effective treatment, preventive and control measures are available
RG4 high individual and community risk (BSL4)	Organism, which may be an exotic agent or new agent usually able to cause life-threatening human disease. The infectious disease is readily transmissible from one individual to another. Infectious disease may be transmitted via aerosol or via an unknown route. Effective treatment, preventive and control measures are not readily available.

Ref: WHO Laboratory Biosafety Manual 2004

APPENDIX 3
Classification of Microorganisms into Risk Groups (RG)

Appendix 3 describes the list of microorganisms according to RG. It is based on existing international norms for the grouping of pathogenic organisms.

Safety considerations in the application of biotechnology are imperative since possible risks in research and development involving different microorganisms have been recognised. According to their possible risks to health and environment, the microorganisms have been classified into different risk groups [Reference: *Prevention & Control of Infectious Diseases (Importation & Exportation of Human Remains, Human Tissues and Pathogenic Organisms & Substances) Regulations, 2006*]. RG1 contains organisms that cause no risk to health and environment (as per definition). However, Good Laboratory Practices have to be followed. The list should ease the grouping and identification of specific strains. It does not compensate the responsibility of the scientists

Note: This is not a complete list. For the purpose of the Prevention and Control of Infectious Diseases Act and Regulations 2007, any organism not listed in RG2, 3 or 4, should not be classified in RG1, until its characteristics and pathogenicity are verified in consultation with the Expert Committee on Prevention and Control of Infectious Disease, Ministry of Health, Malaysia.

BACTERIA: RG1	
• *Acetobacter spp.* • *Actinoplanes spp.* • *Agrobacterium spp.* • *Alcaligenes aquamarines / A.eutrophus / A.latus* • *Aquaspirillum spp.* • *Arthrobacter spp.* • *Azotobacter spp.* • *Bacillus spp., except B.cereus and B.anthracis* • *Bifidobacterium.spp., except B.dentium* • *Brdyrhizobium spp.* • *Brevibacterium spp.* • *Caryphanon spp.* • *Clavibacter spp.* Except *C. michiganensis and C.sepedonicus* • *Clostridium aceticum / C.acetobutylicum / C.acidiurici / C.cellobiparum / C.kluyveri / C.thermoaceticum / C.thermocellum / C.thermosulfurogenes* • *Corynebacterium glutomicum / lilium* • *Enterococcus facium ATCC 4043* • *Escherichia coli ATCC 9637, CCM28, NCIB 8743, B, K12 and derivatives* • *Erwinia spp. Except E.chrysanthemi, E.amylovora and E.herbicola* • *Gluconobacter* • *Klebsiella planticola*	• *Lactobacillus acidophilus / L.bauaricus / L.breuis / L.bucneri / L.casei / L.cellobiosis / L.fermentum / L.ermentum / L.helveticum / L.sake* • *Lactococcus lactis* • *Leuconostoc spp.* • *Lysobacter spp* • *Methanobacter spp.* • *Methylomonas spp.* • *Micrococcus spp.* • *Pediococcus spp.* • *Pseudomonas gladioli / P.fluorescens / P.syringae,* except *P.pathotype persicae* • *Ralstonia spp.* • *Rhizobium spp.* • *Rhodobacter spp.* • *Rhodopseudomonas spp.* • *Staphylococcus carnosus* • *Rickettsiella spp.* • *Streptococcus salivarius-thermophilus* • *Streptomyces spp.,* except *S.somaliensis* • *Thermobacteroides spp.* • *Thermus spp.* • *Thiobacillus spp.* • *Vibrio diazotrophicus / V.fischeri*

BACTERIA, CHLAMYDIA AND MYCOPLASMA: RG2	
• *Acinetobacter baumannii (Acinetobacter calcoaceticus)* • *Acinetobacter lwoffi* • *Actinobacillus actinomycetemcomitans* • *Actinomadura madurae* • *Actinomadura pelletieri* • *Actinomyces spp.* Including: *Actinomyces gerencseriae* *Actinomyces israelii* *Actinomyces pyogenes (Corynebacterium pyogenes)* • *Aeromonas hydrophila* • *Afipia spp* • *Agrobacterium radiobacter* • *Alcaligenes spp.* • *Amycolata autotrophica* • *Archanobacterium haemolyticum (Corynebacterium haemolyticum)* • *Arizona spp* - all serotypes • *Bacillus cereus* • *Bacteroides spp.* Including: *Bacteroides fragilis* • *Bartonella bacilliformis (Rochalimaea bacilliformis)* • *Bartonella quintana (Rochalimaea quintana)* • *Bartonella henselae (Rochalimaea henselae)* • *Bartonella vinsonii (Rochalimaea vinsonii)* • *Bordetella bronchiseptica / Bordetella parapertussis* • *Bordetella pertussis* • *Borrelia spp.* including: *B. burgdorferi / B. Duttonii / B.recurrentis* • *Brucella ovis* • *Fluoribacter bozemanae (*Formerly *legionella)* • *Francisella tularensis (Type B)* • *Fusobacterium spp.* including: *Fusobacterium necrophorum* *Gardnerella vaginalis* • *Haemophilus spp.* including: *Haemophilus ducreyi/* *H. influenzae* • *Helicobacter pylori* • *Klebsiella spp.* including: *Klebsiella pneumonia /* *K. oxytoca* • *Legionella spp.* including: *Legionella pneumophila*	• *Burkholderia spp.* including: *Burholderia cepacia /* *B. mallei (Pseudomonas mallei)* *B. pseudomallei (Pseudomonas pseudomallei)* • *Campylobacter spp.* including: *Campylobacter coli / C. Fetus / C.jejuni* • *Capnocytophaga spp.* • *Cardiobacterium hominis* • *Chlamydia pneumoniae* • *Chlamydia psittaci (non avian strains)* • *Chlamydia trachomatis* • *Citrobacter spp.* • *Clostridium spp.* including: *Clostridium botulinum /* *C. chauvoei / C. Haemolyticum /* *C.histolyticum / C. Novyi / C.perfringens /* *C.septicum / C.tetani* • *Corynebacterium spp.* including: *Corynebacterium diphtheriae /* *C. Minutissimum / C. pseudotuberculosis /* *C. renale* • *Dermatophilus congolensis* • *Edwardsiella tarda* • *Enterobacter spp.* including: *Enterobacter aerogenes /* *E.cloacae* • *Enterococcus spp.* • *Erysipelothrix rhusiopathiae* • *Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive, enterohaemorrhagic and strains bearing K1 antigen, including *E. coli O157:H7 or O103* • *Flavobacterium meningosepticum* • *Nocadia spp.* Including: *Nocardia asteroids /* *N. brasiliensis / N.farcinica /* *N. nova / N.otitidiscaviarum /* *N. transvalensis* • *Pasteurella spp.* including: *Pasteurella multocida (except resistant strains listed in RG3)* • *Peptostreptococcus spp.* including: *Peptostreptococcus anaerobius* • *Plesiomonas shigelloides* • *Porphyromonas spp.* • *Prevotella spp* • *Proteus mirabilis / Proteus penneri* • *Proteus vulgaris* • *Providencia spp.* including:

- *Leptospira interrogans - all serotypes*
- *Listeria ivanovii*
- *Listeria monocytogenes*
- *Moraxella catarrhalis*
- *Moraxella lacunata*
- *Morganella morganii*
- *Mycobacterium spp.* including:
  *(*except *those listed in Risk Group 3)*
  Mycobacterium avium / Intracellulare
- *M. asiaticum M. africanum / M. Chelonei / M. bovis (BCG vaccine strain) / M.fortuitum / M.kansasii / M.leprae / M. Malmoense / M.marinum / M. microti / M.paratuberculosis / M. scrofulaceum / M.simiae / M.szulgai / M.ulcerans / M.xenopi*
- *Mycoplasma caviae / Mycoplasma hominis*
- *Mycoplasma pneumoniae*
- *Neisseria elongata / Neisseria gonorrhoeae*
- *Neisseria meningitidis*
- *Streptococcus spp.* including:
  Streptococcus pneumoniae / S.pyogenes
- *Streptococcus suis*
- *Treponema spp.* including:
  Treponema carateum / T. Pallidum / T.pertenue
- *Ureaplasma urealyticum*
- *Providencia alcalifaciens / P.rettgeri*
- *Pseudomonas aeruginosa*
- *Rhodococcus equi*
- *Rochalimaea spp.* (refer to *Bartonella spp.*)
- *Salmonella spp.* including:
  Salmonella arizonae / S. cholerasuis / S. enteritidis / S. gallinarum-pullorum / S. meleagridis / S. paratyphi, A, B, C S. typhi / S. typhimurium
- *Serpulina spp.*
- *Serratia liquefaciens / Serratia marcescens*
- *Shigella boydii*
- *Shigella dysenteriae* (all serotypes)
- *Shigella flexneri / Shigella sonnei*
- *Sphaerophorus necrophorus*
- *Staphylococcus aureus*
- *Stenotrophomonas maltophilia*
- *Streptobacillus moniliformis*
- *Vibrio spp.* Including:
  Vibrio cholerae / V.parahemolyticus / V.vulnificus
- *Yersinia spp.* (except *Yersenia pestis, listed in Risk Group 3)*
- *Yersinia enterocolitica / Yersenia pseudotuberculos*

BACTERIA, CHLAMYDIA AND RICKETTSIA: RG3

- *Bacillus anthracis*
- *Brucella spp.* (except *B. ovis,* listed in Risk Grp 2):
  Brucella abortus
  Brucella canis
  Brucella melitensis
  Brucella suis
- *Burkholderia (Pseudomonas) mallei*
- *Burkholderia (Pseudomonas) pseudomallei*
- *Chlamydia psittaci (avian strains)*
- *Coxiella burnetii*
- *Ehrlichia spp.* including:
  Ehrlichia sennetsu (Rickettsia sennetsu)
- *Eikenella corrodens*
- *Francisella tularensis* (Type A)
- *Mycobacterium bovis (*except *BCG strain, refer to RG 2)*
- *Mycobacterium tuberculosis* (multi-drug resistant strains)
- *Pasteurella multocida type B -"buffalo" and other virulent strains*
- *Rickettsia spp.* including:
  *Rickettsia akari / R. australis / R. canada/ R. conorii / R. prowazekii / R. rickettsii / R.sennetsu (*refer to *Ehrlichia sennetsu) R. siberica / R. Tsutsugamushi / R. typhi (Rickettsia mooseri)*
- *Yersinia pestis*

BACTERIA, CHLAMYDIA, MYCOPLASMA AND RICKETTSIA: RG4

NONE

VIRUSES: RG1
• Attenuated viral strains which are accepted vaccines. Only a limited number of passages in defined cell-culture or host-systems are allowed • Apathogenic viral strains • Viral strains from fungal or bacterial systems, provided they do not contain virulence-factors and are described as apathogenic for higher animals and human beings • Baculoviruses of insects

VIRUSES: RG2	
• Adenoviridae Adenoviruses, all serotypes • Arenaviridae Lymphocytic choriomeningitis virus complex (LCM); non-neurotropic strains; Ippy, Mobala – Tacaribe virus complex: Ampari, Latino, Parana, Pichinde, Tacaribe, Tamiami • Hepatitis delta virus • Astroviridae Human astrovirus • Bunyaviridae – Genus: Bunyavirus Bunyamwera virus, California encephalitis group, including LaCrosse virus – Genus: Phlebovirus all species, except Rift Valley fever virus (refer to RG3), includes: Rift Valley fever virus vaccine strain MP-12, Sandfly fever virus, Toscana, Uukuvirus • Herpesviridae all Herpesviruses, *except Herpesvirus simiae (refer to RG 4):* Cytomegalovirus Epstein Barr virus Herpes simplex types 1 and 2 Herpes varicella-zoster Human herpesvirus type 6 (HHV 6) Human herpesvirus type 7 (HHV 7) Human herpesvirus type 8 (HHV 8) • Orthomyxoviridae Influenza viruses types A, B, C, *except Avian Influenza A, H5N1, (Refer to RG3)* – Other tick-borne orthomyxoviruses such as Dhori and Thogoto	– Genus: Nairovirus Hazara virus, Dugbe virus • Caliciviridae all isolates including Norwalk virus, Sapovirus and Hepatitis E virus • Coronaviridae Human coronaviruses (serotypes, 229E and OC43) *except SARS coronavirus* (refer to RG 3) • Flaviviridae – Genus: Flavivirus (Group B Arbovirus): Dengue virus serotypes 1, 2, 3, 4, Yellow fever virus vaccine strain 17D, Japanese encephalitis virus – Genus: Hepacivirus Hepatitis C virus • Hepadnaviridae Hepatitis B virus • Parvoviridae – Genus: Parvovirus all isolates including human parvovirus (B19) • Picornaviridae – Genus: Aphthovirus – Genus: Cardiovirus – Genus: Enterovirus Coxsackie virusesA and B Echoviruses, Polioviruses Enterovirus serotypes 68 – 71 – Genus: Rhinoviruses – Genus: Hepatovirus Hepatitis A • Polyomaviridae all isolates including BK and JC viruses, Simian virus 40 (SV 40) • Poxviridae all types, *except Monkeypox virus and restricted poxviruses such as Alastrim, Smallpox, and Whitepox (refer to RG 3 and 4)* includes viruses: Buffalopox, Cowpox, Milker' s nodule, Molluscum,contagiosum, Orf, Vaccinia, Yabapox and Tanapox

• Papillomaviridae – Genus: Papillomavirus all human papilloma viruses • Paramyxoviridae – Genus: Paramyxovirus all isolates including Human parainfluenza viruses types 1, 2, 3 and 4, and Newcastle disease virus – Genus: Pneumovirus all isolates including Respiratory syncytial virus – Genus: Morbillivirus all isolates including measles virus – Genus: Rubulavirus Mumps virus – Genus: Metapneumovirus Human metapneumovirus	• Reoviridae – Genus: Coltivirus all types including Colorado tick fever virus – Genus: Rotavirus all human Rotaviruses – Genus: all isolates of Orthoreovirus and Orbivirus

VIRUSES: RG2 (contd)	
• Rhabdoviridae Genus: Lyssavirus Rabies virus (fixed virus / vaccine strain) – Genus: Vesiculovirus Vesicular stomatitis virus - laboratory adapted strains including VSV-Indiana, San Juan and Glasgow, Piry, Chandipura	• Togaviridae – Genus: Alphaviruses - Group A Arboviruses – Bebaru, Barmah forest virus, Chikungunya, O'nyong-nyong, Ross river virus, Semliki forest virus, Sindbis, Venezuelan equine encephalomyelitis vaccine strain TC-83 only – Genus: Rubivirus Rubella virus

VIRUSES AND PRIONS: RG3	
• Arenaviridae Flexal, Mopeia Lymphocytic choriomeningitis virus (LCM) (neurotropic strains) • Bunyaviridae – Genus: Hantaviruses Hantaan virus (Korean Haemorrhagic fever), Seoul, Sin Nombre virus, Belgrade, Puumala and unclassified Bunyaviruses – Genus: Nairovirus Bhanja – Genus: Phlebovirus Rift Valley fever virus • Coronaviridae SARS Coronavirus • Flaviviridae - Group B Arboviruses – Genus: Flavivirus Yellow fever virus (wild type), West Nile fever virus, St. Louis encephalitis virus, Murray Valley encephalitis virus	– Ntaya virus group: Israel turkey meningitis virus – Modoc virus group: Sal Vieja virus, San Perlita virus – Tentative species: Rocio, Spondweni, Wesselsbron – Tick-borne encephalitis virus group: Hanzalova, Absettarov, Hypr, Kumlinge, Louping Ill, Negishi, Powassan • Orthomyxoviridae Avian Influenza virus A, H5N1 • Retroviridae – Human immunodeficiency virus (HIV) types 1 and 2 – Human T cell lymphotropic virus (HTLV 1 and 2) – Simian immunodeficiency virus (SIV) • Rhabdoviridae Rabies virus (Street virus)

• Unclassified Viruses Chronic infectious neuropathic agents (CHINA) – Prions – Transmissible spongioform encephalopathies (TME) agents: – Bovine spongiform encephalopathy (BSE), Creutzfeldt-Jacob disease (CJD), Variant Creutzfeldt- Jacob disease, Fatal familial insomnia, Gerstmann-Straussler-Scheinker syndrome and Kuru	

VIRUSES: RG4	
• Arenaviridae – Genus: Arenaviruses Lassa, Guanarito, Junin, Machupoand Sabia • Bunyaviruidae – Genus: Nairovirus Crimean-Congo hemorrhagic fever virus • Filoviridae all Ebola viruses and ;Marburg virus • Flaviridae (Togaviruses) - Group B Arboviruses – Tick-borne encephalitis virus complex including Central European encephalitis Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses – Herpesviruses (alpha) Herpesvirus simiae (Herpes B or Monkey B virus)	• Poxviridae – Variola major, variola minor, whitepox, alastrim (Importation of organisms, including alastrim, smallpox (variola) and whitepox is strictly prohibited. All activities, including storage of variola and whitepox, are restricted to a single facility (World Health Organisation Collaborating Center for Smallpox Research, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America). – Hemorrhagic fever agents and viruses as yet undefined

PARASITES: RG2	
• *Acanthamoeba spp.* • *Ancylostoma human hookworms* including: *Ancylostoma duodenale* *Ancylostoma. Ceylanicum* • *Angiostrongylus spp.* • *Anisakis simplex* *Ascaris* including *Ascaris lumbricoides, Ascaris suum* • *Babesia* including: *Babesia divergens, Babesia microti* • *Balantidium coli* • *Blastocystis hominis* • *Brugia filaria* worms including: *Brugia malayi, Brugia timori* • *Capillaria spp.* • *Coccidia* • *Contracaecum osculatum* • *Cryptosporidium spp.* including: *Cryptosporidium parvum*	• *Giardia spp.* including: *Giardia lamblia (Giardia intestinalis)* • *Heterophyes spp.* • *Hymenolepis diminuta / Hymenolepis nana* • *Isospora belli* • *Leishmania spp. (*mammalian) except *Leishmania braziliensis and Leishmania donovani (Refer to Risk Group 3)* includes: – *Leishmania ethiopia,Leishmania major, Leishmania mexicana, Leishmania peruvania, Leishmania tropica* – *Loa loa* filaria worms • *Mansonella spp.* such as: *Mansonella ozzardi,* *Mansonella perstans,* *Mansonella streptocerca* • *Metagonimus spp.* • *Microsporidium spp.*

• *Cyclospora spp* including: *Cyclospora cayetanensis* • *Cysticercus cellulosae (*hydatid cyst, larva of *Taenia solium)* • Dicrocoelium dendriticum • Dientamoeba fragilis • Dracunculus medinensis • Entamoeba histolytica • Enterobius vermicularis Enterocytozoon bieneusi Fasciola gigantica */ Fasciola hepatica, Fasciolopsis buski* • *Paragonimus spp* including: *P. westermani* • *Plasmodium spp.* (human and simian) including: *Plasmodium cynomologi, Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax* • *Sarcocystis suihominis* • *Schistosoma spp.* including: *Schistosoma haematobium, Schistosoma intercalatum, Schistosoma japonicum, Schistosoma mansoni, Schistosoma mekongi* • *Strongyloides spp.* including: *Strongyloides stercoralis* *Taenia saginata / Taenia solium* • *Toxocara spp.* including: *Toxocara canis*	• *Naegleria spp.* • *Necator* human hookworms including: *Necator. americanus* • *Onchocerca* filaria worms including, *Onchocerca volvulus* • *Opisthorchis felineus* *Opisthorchis sinensis (Clonorchis sinensis)* • *Opisthorchis viverrini (Clonorchis viverrini)* • *Toxoplasma spp.* including: *Toxoplasma gondii* • *Trichomonas vaginalis* • *Trichinella nativa / Trichinella nelsoni / Trichinella pseudospiralis / Trichinella spiralisTrichostrongylus spp.* Including *Trichostrongylus orientalis* • *Trichuris trichiura* • *Trypanosoma brucei sub-spp. except Trypanosoma brucei rhodesiense and Trypanosoma cruzi (Refer to RG 3)* includes *Trypanosoma brucei gambiense* • *Wuchereria bancrofti* filaria worms

PARASITES: RG3	
• *Echinococcus spp. such as: Echinococcus granulosis, Echinococcus multilocularis, Echinococcus vogeli* • *Leishmania braziliensis, Leishmania donovani*	• *Trypanosoma brucei rhodesiense* • *Trypanosoma cruzi*

PARASITES: RG4
NONE

FUNGI: RG1	
Agaricus bisporus *Acremonium chrysogenum / strictum / elegans* *Actinomucor elegans* *Ashyba gossypii* *Aspergillus oryzae* *Aureobasidum pullulans* *Blakeslea trispora* *Brettanomyces bruxellensis* *Candida boindinii / shehateae / utilis* *Chaetonium globosum* *Cladosporium cladosporioides* *Claviceps paspali / purpurea* *Coprinus cinereus* *Cunninghamella blakesleana / elegans* *Curvularia lunate* *Cyathus stercoreus* *Debaryomyces hansenii* *Dacrymyces deliquescens* *Engyodontium album* *Geotrichum candidum* *Hansenula anomala / polymorpha* *Hypholama fasciculare / roseonigra* *Engyodontium album* *Geotrichum candidum* *Hansenula anomala / polymorpha* *Hypholama fasciculare / roseonigra* *Kloecdera corticis* *Lentinus edodes* *Lipomyces lipofer / sarkeyi* *Metarhizium anisopliae* *Monascus pupureus / ruber* *Moniliella suaveolena* *Mortierella vinacea*	*Mucorcircinelloides / mucedo / plumbeus / rouxii* *Myrothecium verrucaria* *Neurospora crassa/sitophilla* *Nigrospora sphaerica* *Oxyporus populinus* *Pachysolen tannophilus* *Paecilomyces varioti / lilacinus* *Penicillium camemberti / chrysogenum / funiculosum* *Phycomyces blakesleanus* *Pichia farinosa / guilliermondii / membranae faciens / stipitis* *Pleurotus ostreatus* *rhizoctonia solani* *Rhizopus oryzae / stolonifer* *Rhodosporidum toruloides* *Rhodotorual glutinis* *Saccharomyces cerevisiae* *Schizoasccharomyces pombe* *Schwanniomyces occidentalis* *Sordaria macrpsopra* *Thanatephorus cucumeris* *Trametes vesicolor* *Trichoderma harzianum / longibrachiatum / viridae* *Trigonopsis variabilis* *Verticillium lecanii* *Volvariella volvacea* *Wallernia sebi* *Xeromyces bisporus* *Zygorhynus moelleri* *Zygosaccharomyces bailii / rouxii*

FUNGI: RG2	
• *Asperigillus fumigatus* • *Asperigillus flavus* • *Candida albicans* • *Candida tropicalis* • *Cryptococcus neoformans var neoformans (Filobasidiella neoformans var neoformans)* • *Cryptococcus neoformans var gattii (Filobasidiella bacillispora)* • *Dactylaria galopava (Ochroconis gallopavum)* • *Emmonsia parva var parva* • *Emmonsia parva var crescens* • *Epidermophyton spp.* including: *Epidermophyton floccosum*	• *Exophiala (Wangiella) dermatitidis Fonsecaea compacta / Fonsecaeapedrosoi* • *Madurella grisea* • *Madurella mycetomatis* • *Microsporum spp* • *Neotestudina rosatii* • *Penicillium marneffei* • *Scedosporium apiospermum (Pseudallescheria boydii)* • *Scedosporium proliferans (inflatum)* • *Sporothrix schenckii* • *Trichophyton spp.* including: *Trichophyton rubrum*

FUNGI: RG3	
• *Blastomyces dermatitidis (Ajellomyces dermatitidis)* • *Cladophialophora bantiana (Cladosporium bantianum, Xylohypha bantiana)* • *Cladosporium trichoides* • *Coccidioides immitis*	• *Histoplasma capsulatum spp.* including: – *Histoplasma capsulatum var capsulatum* – *Histoplasma capsulatum va farcinimosum* – *Histoplasma. capsulatum var. duboisii* • *Paracoccidioides braziliensis*

FUNGI: RG4
NONE

APPENDIX 4
Host/Vector Systems Providing Biological Containment

The objective of biological containment is to minimise both the survival of the host and vector outside the laboratory, and the transmission of the vector from the propagation host to a non-laboratory host. This Appendix lists the host/vector systems which are currently accepted as providing a level of biological containment (Singapore Biosafety Guidelines for Research on GMOs 2006).

	HOST	VECTOR
BACTERIA	• *Escherichia coli* K12 or *E. coli* B derivatives which do not contain conjugative or generalized transducing phages • *Bacillus subtilis* or *B. licheniformis* • Asporogenic strains with a reversion frequency of less than 10 -7 • *Pseudomonas putida* Strain KT 2440 • *Streptomyces* specified species – *S. coelicolor, S. lividans, S. parvulus, S. griseus*	• Non-conjugative plasmids • Bacteriophage – lambda – lambdoid – Fd or F1 (e.g.M13) • Indigenous *Bacillus* plasmids and phages whose host range does not include *B. cereus* or *B. anthracis* Certified plasmids: pKT 262, pKT 263, pKT 264 Certified plasmids: SCP2, SLP1, SLP2, PIJ101 and derivatives Actinophage phi C31 and Derivatives
FUNGI	• *Neurospora crassa*, laboratory strains *Saccharomyces cerevisiae* *Pichia pastoris* *Schizosaccharomyces pombe*	No restriction
SLIME MOULDS	*Dictyostelium* species	*Dictyostelium* shuttle vectors, including those based on the endogenous plasmids Ddp1 and Ddp2
TISSUE CULTURE	• Mammalian (including human) cells • Avian cells • Plant cell cultures • Insect cell cultures such as *Spodoptera frugiperda*	• Non-viral vectors or defective viral vectors (including retrovirus or retroviral-helper combinations) that cannot infect human cells. • Avipoxvirus vectors • Non-tumorigenic disarmed Ti plasmid vectors in *Agrobacterium tumefaciens* and non-pathogenic viral vectors* * Baculovirus (*Autographa californica* nuclear polyhedrosis virus)

Note: The above approved hosts may also be used in experiments where DNA is inserted into the host cell without the use of a biological vector (for example, by mechanical, electrical or other means), provided that the DNA:

- Is not derived from microorganisms able to cause disease in humans, animals or plants, unless the DNA to be introduced is fully characterized and will not increase the virulence of the host or vector
- does not code for a toxin for vertebrates with an LD50 of less than 100 ˜g/kg, and is not an oncogene
- does not comprise or represent more than two-thirds of the genome of a virus and Is not being used in an experiment in which the genetic material missing from the viral genome and essential for producing infection is available in the cell into which the incomplete genome is introduced, or is made available by subsequent breeding processes
- Any commercially available Host-Vector Systems. Such a system with an approved host and the DNA meeting these conditions would constitute an approved host/vector system for the purposes of these *Guidelines*
- Any other plants expression vectors available either derived from *Agrobacterium* species or other commercially available vector systems

APPENDIX 5
Quarantine Pests of Malaysia

Pest	Common Name
Avocado (*Persea* spp.)	
Sunblotch virus of avocado	
Banana (*Musa* spp.)	
Bunchy top virus	-
Mycoplasma-like organism	Cameroon Marbling disease
Mycosphaerella musicola var difformis	Black sigatoka
Pseudomonas solanacearum	Moko disease
Barley (*Sorghum* spp)	
Barley stripe mosaic	
Cassava (*Manihot esculenta*)	
Caliothrips masculinus	Thrip
Cassava brown streak virus	Brown streak
Cassava witch's broom	Witch's broom
Cassave mosaic virus	Mosaic
Monomychelus tanajoa	Tanajoa or green cassava mite
Mycoplasma-like organism	Witch's broom
Oligonychus peruvianus	Mite
Phaeolus manihotis Hein	Root rot
Sphaceloma manihotis	Super elongation disease
Super elongationdisease	-
Unknown	African mosaic
Unknown	Cassava latent virus disease
Virus	Frog's skin
Citrus (*Citrus spp.*)	
Anastrepha fraterculus (Wied)	-
Anastrepha ludens	Mexican fruit fly
Anastrepha mombinpraeoptans	West Indian fruit fly
Anastrepha spp.	Fruit flies
Citrus greening	Mycoplasma
Citrus stubborn disease	Stubborn disease
Creatitis capitata (Weid)	Mediterranean fruit fly
Creatitis rosa Karsh	Natal fruit fly
Dacus tryoni (Frogg)	Queensland fruit fly
Deuterophoma tracheiphila Petri	Mal secco
Exocortis virus	Exocortis
Impietratura virus	Impietratura
Quadraspidiotus perniciosus	San Jose scale
Satsuma dwarf virus	Satsuma
Spiroplasma citri	Stubborn disease
Xyloporosis virus	Xyloporosis
Yellow virus	Shell bark

Cocoa (*Theobroma cocoa*)	
Acrocercops cramerella	Cocoa pod borer
Bathycoella thalassina	-
Cacao red mottle virus	Red mottle
Cacao swollen shoot virus complex	Swollen shoot
Cacao vein-clearing virus	Vein clearing
Cocoa yellow mosaic virus	Yellow mosaic
Distantiella theobroma Dist.	Capsid bug causing dieback
Helopeltis bergrothis Rent.	Capsid bug causing canker
Marasmius perniciosus Stahel	Witch's broom
Monalonium sp.	Mirid bug
Monilia roreri Cif	Monilia pod rot
Oncobasidium theobromae	Vascular streak dieback
Phytophthora palmivora	Balck pod
Sahlbergella singularis Haghl.	Capsid bug causing dieback
Stenoma decora Zeller	Cacoa fruit & shooter borer
Trachysphaera fructigena Tabor et Bunting	Trachysphaera pod rot
Virus	Water mark
Virus	Yellow vein banding
Coconut (*Cocos nucifera*)	
Artona catoxantha	Leaf moth
Awka (Nigeria)	Cause unknown
Cadang-cadang	Viroid
Cape St. Paul wilt (Ghana)	Cause unknown
Coelaenomenodera elaeidis Maylik	Leaf miner
Eriophyes guerreronis	Mite
Kaincope (Togo)	Cause unknown
Kribi (Cameroons)	Cause unknown
Lethal yellowing	Mycoplasma
Marasmiellus cocophilus	Lethal bole rot
Melitomma insular Frm.	Wod borer
Mycoplasma-like organism	Tanzania disease
Natuna wilt	Cause unknown
Oryctes boas	Beetle
Oryctes monocerus	Beetle
Oryctes rhinoceros	Rhinoceros beetle
Pachymerus nucleorum (F)	Coconut borer
Phytophthora heveae	Phytophthora rot
Phytophthora palmivora	Crown rot
Pseudotheranthus devastans Dist.	Coreid bug
Pseudotheranthus wayi Br.	Coreid bug
Rhadinaphelenchus cocophilus (Cobb)	Red ring disease
Rhynchophorus palmarum	Palm weevil
Rickettsiae	Decline disease
Setora nrtens	Nettle caterpillar
Tapipaka/Coconut wilt	Cause unknown
Unknown	Head droop
Unknown	Leaf scorch
Unknown	Little leaf
Unknown	Malaysian wilt
Unknown	Soccoro wilt

Coffee (Gossypsium spp.)	
Anthonomus grandis Boh	Mexican cotton boll weevil
Anthonomus spp.	Boll weevils
Anthonomus vestitus Boh	Peruvian cotton boll weevil
Cotton leaf curl virus	Leaf curl
Phymatotrichum omnivorum (Shear) Dunggar	Texas root rot
Sacadedes pyralis Dyar	False pink boll-worm
Grape (*Vitis Vinifera*)	
Agrobacterium tumefaciens	Crown ball
Bacteria	Pierce's disease
Fan leaf virus	Fan leaf, yellow mosaic and vein banding
Guignardia bidwellii	Black rot
Leaf-roll virus	Leaf-roll
Mycoplasma-like organism	Flavescene doree
Nepo viruses (Grapevine fanleaf virus and its strains, Arabis mosaic, Hungarian chrome mosaic virus, Raspberry ring spot virus)	
Virus	Grapevine corky bark
Virus	Grape 'legno riccio' or stem pitting
Xanthomonas ampelina	Bacterial blight or necrosis
Groundnut (*Arachi hypogaea*)	
Marginal chlorosis virus	Marginal chlorosis
Peanut stunt virus	Stunt
Phymatotrichum omnivorum	Root rot
Rosette viruses	Rosette disease
Sphaceloma arachidis	Scab
Verticillium dahliae	Verticillium wilt or pod rot
Hevea Rubber (*Hevea brasiliensis*)	
Aleurodicus cocois	White fly
Catacauma huberi	Black crust
Erinnyis ello	Sphingid moth
Leptopharsa heveae Drake	Lace bug
Microcyclus ulei P.Henn	South American Leaf Blight
Pellicularia filamentosa	Target leaf spot
Phytophthora spp.	Leaf fall & leaf wither
Premolis semirufa	-
Thanatephorus cucumeris syn	-
Honey Bees (*Apis spp.*)	
Acarapis woodi	Mite
Bacillus larvae	American foulbrood
Nosema apis	Nosema disease
Streptococcus pluton	Euperean foulbrood
Virus	Sacbrood
Mango *(Mangifera indica)*	
Elsinoe mangifera	Mango scab
Noorda albizonalis	Mango seed borer
Stenochetus mangifera	Mango seed weevil
Unknown	Mango malformation and bunchy top
Unknown	Woody gall & scaly bark

Maize (*Zea mays*)	
Claviceps gigantea	Ergot
Corn stunt spiroplasma	-
Diatraea spp. Esp. *D. saccharalis* (F)	Stalk borers
Dreschlera maydis	Corn leaf blight
Erwinia stewartii (E.F. Smith) Dye.	Bacterial wilt
Maize chlorotic dwarf virus	-
Maize dwarf mosaic virus	-
Maize streak virus	-
Maize stripe virus	-
Maize stunt virus	-
Peronosclerospora philippensis	Downy mildew
Prostephanus truncatus	Larger grain borer
Sesamia cretica Led.	Durra stem borer
Oil Palm (*Elaeis guineensis*)	
Cerospora elaeidis Stey	Freckle
Coelaenomenodera elaedis	Leaf miner
Darna trema	Nettle caterpillar
Fusarium oxysporum Schlect	Pusarium wilt
Ganoderma lucidum	Basal stem and root rot
Leptopharsa gibbicarina	Lace bug
Mahasena corbetii	Bag worm
Metisa plana	Bag worm
Oryctes rhinoceros	Rhinoceros beetle
Pachymerus lacerdae (Chevr.)	Kernel borer
Pachymerus nucleorum (F)	Kernel borer
Phytomonas staheli	Marchitez sorpresiva
Pimelephila ghesquierii Tams	Pyralid moth
Retracrus elaeis	Eriophyrid mite
Rhadinaphelenchus cocophilus	Red ring disease
Rhynchophoris phoenicis	Weevil
Setora nitens	Nettle caterpillar
Sibine fusca	Leaf eating caterpillar
Unknown	Fatal yellowing
Unknown	Leaf mottle
Viroid	Cadang-cadang
Orchids	
Cymbidium mosaic virus	Cymbidium mosaic
Orchid mosaic virus	Cattleya flower break
Papaya (*Carica papaya*)	
Papaya bunchy top virus	Bunchy top
Papaya mosaic virus	Papaya mosaic dieback
Papaya ring spot virus	Papaya ring spot and others
Pepper (*Piper nigrum*)	
Cronartium hakensii	Western globoid stem rust
Cronartium ribicola	White pine blister rust
Dothistroma pini	Needle caste
Fusarium moniliforme var subglutinans	Pitch canker
Scirrhia acicola	Needle blight
Scleroderris abietina	Needle twig blight

Potato (*Solanum tuberosum*)	
Corynebacterium sepedonicum	Bacterial ring rot
Ditylenchus destructor	Potato rot nematode
Heterodera rostochiensis	Golden nematode
Leptinotarsa decemlineata	Colorado beetle
Oospora pustulans	Skin spot
Snychytrium endobioticum	Black warf
Rice (Oryza sativa)	
Aphelenchoides besseyi	White tip
Diatraea spp.	Stalk borers
Ephelia pallida	Panicle disease
Fusarium moniliforme var. subglutinans	Bakanae disease
Lissorhopterus oryzaephilus	Rice water weevil
Orseolia oryzae	Rice gall midge
Rice dwarf virus	Dwarf
Rice Hoja blanca virus	White leaf (Hoja blanca)
Rice stripe virus	Stripe
Rice waika virus	-
Sagatodes oryzicola and Cubana Virus	- Transitory yellowing
Silkworm (*Bombyx mori*)	
Nosema bombycis	Pebrine disease
Viruses	Infectious flacherie, grasserie
Soyabean (*Glycine max*)	
Cercospora kikuchii	Purple blotch
Colletotrichum truncatum	Anthracnose
Peronospora manshurica	Downy mildew
Pseudomonas glycinea	Bacterial blight
Pseudomonas tabaci	Wild fire
Virus	Soya bean dwarf virus
Virus	Soya bean mosaic virus
Sugarcane	
Clemonra smithi (Arr.)	Sugarcane grub
D. saccharalis (F.)	Stalk borer
Diantraea spp.	Stalk borer
Diaprepes abbreviates	Sugarcane root stalk borer
Fiji disease	-
Grassy shoot	-
Streak virus	-
Unknown	Ring mosaic
Ustilago scitaminea	Smut
Virus	Dwarf virus
Virus	Sugarcane mosaic
Xanthomonas albilinean (Ashby) Down	Leaf scald
Xanthomonas rubilineaus	Red stripe
Xanthomonas rubrisubalicans	Mottled stripe
Xanthomonas vasculorum	Gumming disease
Sunflower (*Helianthus* spp.)	
Sunflower mosaic virus	Sunflower mosaic
Stone fruit	
Stone fruit virus	

Sweet potato (*Ipomoea batatas*)	
Euscepes postfasciatus	West Indian sweet potato weevil
Sweet potato internal cork virus	Internal cork
Sweet potato mosaic virus	Mosaic
Sweet potato dwarf virus	Dwarf
Tea (*Camellia sinensis*)	
Exobasidium reticulatum	Phloem necrosis virus
Virus	-
Tobacco *(Nicotiana* spp.*)*	
Ephestia elutella	Tobacco moth
Pseudomonas tabaci (Wolf Foster)	Wildfire
Peronospora tabacina Adams	Blue mold
Tung (*Aleurites fordii*)	
Popillia japonica (Newn.)	Japanese beetle
Quadraspidiotus perniciosus (Comst.)	San Jose scale
Septobasidium aleuritidis	Branch canker
Trogoderma granarium Ev.	Khapra beetle
Weeds	
Aeginetia indica	-
Alternanthera philoxeroides	Alligator weed
Baccaris halimifolia	Groundsel bush
Chondrilla juncea	Skeleton weed
Christisonia wightii	-
Mimosa pigra	Giant sensitive plant
Myriophyllum brasiliense	Parrot feather
Oryza barthii	-
Oryza longistaminata	-
Oryza punctata	-
Parthenium hysterophorus	Congress weed
Pennisetum polystachyon	Mission grass
Rottboellia exaltata	Itch grass
Striga angustifolia	Witch weed
Striga densiflora	Witch weed
Striga gesnerioides	Witch weed
Striga hermonthica	Witch weed

Note: This is not a complete list. Please refer to Department of Agriculture Malaysia for more details.

GLOSSARY

1. **Amino Acid** – Amino Acids are the molecular units of organic compounds that make up proteins.

2. **Bacterium** – one of a group of one-celled microorganisms having round, rodlike, spiral or filamentous bodies that are enclosed by a cell wall or membrane and which lack fully differentiated nuclei.

3. **Biohazard** – Hazard is an event or process that is potentially destructive to other physical or natural things or organisms. Biohazard is hazard to humans or the environment resulting from a living or biologically derived material or biological agents or conditions.

4. **Biosafety** – the policies and procedures adopted to ensure the environmentally safe application of biotechnology.

5. **Biotechnology** – any technique that uses living organisms or substances from these organisms to make or modify a product, to improve plants or animals, or to develop microorganisms for specific uses.

6. **Biological Safety Officer (BSO)** – Under IBC Guidelines there may be a designated BSO at the institute level who will be responsible for ensuring and implementing the issues of biosafety at the institute level.

7. **Cell** – the smallest component of life. A membrane-bound protoplasmic body capable of carrying on all essential life processes. A single cell unit is a complex collection of molecules with many different activities.

8. **Competent Authority** – A person/organisation who has acquired through training, qualifications or experience, or a combination of these, the knowledge and skills enabling that person/organisation to perform a specified task and is recognised as competent in that task by government agencies or bodies

9. **Contained use** – any operation, undertaken within a facility, installation or other physical structure, which involves GMOs/LMO that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment.

10. **Containment** – act of restricting or preventing the spread, leak or escape of an experimental object.

11. **Decontamination** – process of removing, destroying or reducing the activity of materials such as toxic chemicals, pathogenic microorganisms, etc. that could endanger an individual or the environment.

12. **Donor organisms** – the organism from which genetic material is obtained for transfer to the recipient organism.

13. **Environment** – humans and their surroundings including the earth's sub-surface.

14. **Export** – intentional trans-boundary movement from one country to another country.

15. **Gene** – the fundamental physical and functional unit of heredity, the portion of a DNA molecule that is made up of an ordered sequence of nucleotide base pairs that produce a specific product or has an assigned function.

16. **Host-vector (HV) system** – a microbial strain (host) and its compatible DNA carrier(s) (vector). The host may be a strain of the bacterium *Escherichia coli* or *Bacillus subtilis*, the yeast *Saccharomyces cerevisiae* or other such organisms that have been genetically manipulated to allow the multiplication and expression of the vector. The vector may be a plasmid, a bacteriophage or a virus, and other carriers of genetic materials all designed to carry readily selectable marker(s) and unique restriction sites for inserting DNA segments.

17. **Import** – intentional transboundary movement GMOs/LMO into Malaysia/one country from another country.

18. **Laboratory supervisor** – a senior reseacher in charge of a laboratory whoestablishes policies on specific entry requirements for personnel who are allowed to enter the laboratory or animal rooms. (e.g. immunisation), develops laboratory procedures and provides advice on potential hazard to persons using the laboratory.

19. **Living Modified Arthropods (LMAs)** – arthropod that are LMO

20. **Living Modified Organism (LMO)** – any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology. The term GMO or LMO are used interchangeably to denote the same thing pertaining to modern biotechnology.

21. **Modern Biotechnology** – application of in vitro nucleic acid techniques, including recombinant DNA and direct injection of the nucleic acid into cells or organels; or fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers that are not technique used in traditional breeding and selection.

22. **Move (moving, movement)** – to ship, offer for shipment, offer for entry, import, receive for transportation, carry, or otherwise transport or allow to be transported into, through, or within Malaysia.

23. **Organism** – any active, infective, or dormant stage or life form of an entity characterised as living, including plants, bacteria, fungi, mycoplasmas, mycoplasma-like entities, vertebrate and invertebrate animals, as well as entities such as viroids, viruses, or any living entity related thereto.

24. **Pathogen** – a disease-causing organism.

25. **Person** – any individual, partnership, corporation, company, society, association, or other organised group.

26. **Pest** – any living stage (including active and dormant forms) of insects, mites, nematodes slugs, snails, protozoa, or other animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; other plants and animals that can damage aquatic and terrestrial ecosystems; or any infectious agents or substances which can directly or indirectly injure or cause disease or damage to humans, plant or animals or any processed, manufactured, or other products of plants or animals.

27. **Phage** – eating or destroying characteristic of a bacterial virus.

28. **Plant** – any living stage or form of any member of the plant kingdom including, but not limited to, eukaryotic algae, mosses, club mosses, ferns, angiosperms, gymnosperms, and lichens (which contain algae) including any parts (e.g. pollen, seeds, cells, tubers, stems) thereof and any cellular components (e.g. plasmids, ribosomes, etc.). Plant takes nutrient in soluble form.

29. **Plasmid** – a self-replicating, circular, extra-chromosomal DNA molecule.

30. **Recipient organism** – the organisms that receives genetic material from a donor organism.

31. **Recombinant DNA (rDNA)** – a DNA molecule into which a foreign DNA has been inserted.

32. **Release into the environment** – any intentional introduction of living modified organisms or products of such organisms into the environment through the activities or for the purposes specified in the Second Schedule of Biosafety Act.

33. **Species** – reproductive communities and populations- that are distinguished by their collective manifestation of ranges of variations with respect to many different characteristics and qualities. Species is also a group of very similar organisms which breed and produce fertile offsprings. Different species may breed but do not produce fertile offsprings.

34. **Tissue culture** – the propagation of tissue removed from organisms in a laboratory environment that has strict sterility, temperature and nutrient requirements,

35. **Transgenic animals or plants** – animals or plants whose hereditary DNA has been augmented by the addition of DNA from a source other than parental germplasm, in a laboratory using recombinant DNA techniques.

36. **Vector** – a carrier or transmission agent. In the context of recombinant-DNA technology, a vector in the DNA molecule used to introduce foreign DNA into host cells. Recombinant-DNA vectors include plasmids, bacteriophages, and other forms of DNA.

37. **Vector or vector agent** – organisms or objects used to transfer genetic material from the donor organism to the recipient organism.

ABBREVIATIONS

BSL : Biosafety Level

BSO : Biological Safety Officer

EPA : Environmental Protection Agency

GLP : Good Laboratory Practices

GM : Genetically Modified

HEPA : High Efficiency Particulate Air

IATA : International Air Transport Association

IBC : Institutional Biosafety Committee

LMO : Living Modified Organisms

MAQIS : Malaysian Quarantine Inspection Services

NBB : National Biosafety Board

PI : Principal Investigator

PPE : Personal Protective Equipment

RG : Risk Group