Royal North Shore Hospital

Laboratory Procedures Manual for the handling of Genetically Modified Organisms

Version 1.12

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Acknowledgements

This Manual is based on the PC2 Laboratory Procedures Manual developed for the Kolling Institute by Vic Danis. The material is largely derived from published OGTR Guidelines and from the chapter on “Biological Safety” in the CCH Laboratory Safety Manual.

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SCOPE OF THE MANUAL

This manual is intended to provide guidelines for the use of genetically modified organisms (GMOs see Appendix 3 for glossary of acronyms) in certified laboratories of the Royal North Shore Hospital and in the Gore Hill Animal House. It does not cover requirements for PC2 Plant or Insect Containment facilities, nor for facilities higher than PC2. Its purposes are to:

- Outline the internal operational procedures to be used in relation to the management of GMOs
- Outline procedures for dealing with accidents and incidents with GMOs
- Provide training for staff in the use of GMOs

Governing legislation

These Guidelines are issued in accordance with section 90 of the *Gene Technology Act 2000* (the Act) and set out the requirements for the certification of facilities to specified containment levels.

The Act and the accompanying *Gene Technology Regulations 2001* (the Regulations) form part of the national scheme for the regulation of gene technology and GMOs in Australia.

The objectives of the Act are to:

- Protect the health and safety of people (which includes facility workers and the general public); and
- Protect the environment.

The Act aims to fulfil these objectives by identifying risks posed by, or as a result of, gene technology and by managing those risks through regulating dealings with GMOs. The Regulations complement the Act and provide additional information to assist the interpretation and operation of the provisions in the Act. The Act establishes a statutory officer, the Regulator, who is responsible for deciding on applications for licences. The OGTR is responsible for administering the Act and the Regulations.

Purpose of Certification

Certain dealings with GMOs must be conducted within physical containment facilities. The purpose of certification is to satisfy the Regulator that the containment facility protects persons outside the facility from exposure to GMOs and prevents release of GMOs into the environment. The Regulator also imposes conditions of certification that require certain procedures to be followed to ensure the safety of people working inside containment facilities with GMOs.

Purpose of the Guidelines

These Guidelines detail technical and procedural requirements which are the criteria that must be met by a facility before it is certified, and which need to be met continually to maintain the certification.

A licence to conduct dealings with GMOs may require compliance with these Guidelines. Dealings that are notifiable low risk dealings (NLRD), as defined in the Regulations, are required to be conducted within facilities certified by the Regulator at PC2 or above unless written permission has been obtained from the Regulator.

The Guidelines only include requirements that contribute to achieving the objectives of the Act. They do not provide a comprehensive coverage of biosafety, laboratory safety or broader occupational health and safety issues.

FOUR PHYSICAL CONTAINMENT LEVELS

The Guidelines for Certification of Facilities defines three levels of physical containment for laboratory work involving genetic manipulation. These were previously referred to as Cl, C2 and C3, with C3 being the highest level of containment.

These physical containment levels are now defined under the Standards Australia classification set out in AS/NZS 2243.3:1995 *Safety in laboratories - Microbiology*. This standard sets out four physical containment levels for laboratory work with microorganisms. It details requirements and recommendations for facilities, protective equipment, training and supervision, and work practices in laboratories where work with microorganisms is carried out. The physical containment levels are:

- Physical Containment Level 1 (PC1) - This level of facility with its practices and equipment is appropriate for student and undergraduate teaching and for food industry laboratories. It is suitable for work with microorganisms where the hazard levels are low, and where laboratory personnel can be adequately protected by standard laboratory practice.
- Physical Containment Level 2 (PC2) - This level applies to clinical, diagnostic, industrial, teaching and other premises where work is carried out with indigenous, moderate-risk agents present in the community, and which may be associated with animal, plant or human disease of moderate severity. Diagnostic samples, human blood, and body fluids from humans or animals would normally be regarded as Risk Group 2 and must be handled in PC2 facilities. If a microbial pathogen is isolated from, or suspected to be present in, a specimen, the specimen must be handled according to the requirements of the corresponding risk group, and at the appropriate physical containment level.
- Physical Containment Level 3 (PC3) - This level applies to clinical, diagnostic and other premises where work is carded out with indigenous or exotic agents where there is a risk of serious infection to plants, animals or humans.
- Physical Containment Level 4 (PC4) - This level of facility with its practices and equipment is applicable to work with dangerous agents that pose a high individual risk of life-threatening disease and may be readily spread in the community.

AS/NZS 2243.3 also classifies microorganisms into risk groups numbered from one to four, one being the least hazardous (see *Appendix 1*). These risk groups correspond to the physical containment levels. This means that, for example microorganisms from Risk Group 1 can be handled in PC1 level facilities and up; the minimum requirements for facilities handing Risk Group 2 microorganisms are PC2 level and up, and so forth. For more detail on the classification of microorganisms and physical containment levels for facilities working with microorganisms.

Practices and procedures for the different containment levels are cumulative. That is, all the requirements of the levels below apply to higher levels with more stringent conditions being added at higher levels. Therefore all the requirements of PC1 laboratories are also applicable to PC2 laboratories. All work done in a laboratory of a specific level must follow all the procedures prescribed for that level of physical containment.

GENETIC MANIPULATION WORK

Introduction

The work that can be conducted in a facility that is certified as a Physical Containment Level 2 (PC2) Laboratory Facility includes work with GMOs that present only a moderate potential risk to people and/or the environment, some work with plant tissue culture, and some small scale work with GM animals. It should not, however, include the housing of animals in the facility or the growing of whole plants in the facility.

National scheme

A new national scheme for the regulation of genetically modified organisms (GMOs) commenced in June 2001. A national regulatory package, together with corresponding legislation to be enacted in each State and Territory in Australia, will comprise the national scheme. National regulatory requirements for the new scheme are set out in three Acts, subordinate legislation and other instruments. The Federal Government passed the three relevant Acts - the *Gene Technology Act 2000*, the *Gene Technology (Consequential Amendments) Act 2000* and the *Gene Technology (Licence Charges) Act 2000* - in December 2000. They came into force on 21st June 2001. The legislation is the Commonwealth Government's component of the national scheme.

The *Gene Technology (Licence Charges) Act 2000* provides for regulations to be made establishing annual charges to be paid by the holder of a GMO licence. The regulations will take effect from 21 June 2003.

Defining a GMO

The *Gene Technology Act 2000* (GT Act) defines a genetically modified organism (GMO) as:

(a) an organism that has been modified by gene technology; or

(b) an organism that has inherited traits from an organism (the 'initial organism), being traits that occurred in the initial organism because of gene technology; or

(c) anything declared by the regulations to be a genetically modified organism, or that belongs to a class of things declared by the Regulations to be genetically modified organisms;

The term does not include:

- a human being, if the human being is covered by point (a) only because the human being has undergone somatic cell gene therapy; or
- an organism declared by the Regulations not to be a genetically modified organism, or that belongs to a class of organism declared by the Regulations not to be genetically modified organisms.

To understand the definition of GMO, it is important to clarify the terms "organism" and "gene technology". "Organism" is defined in the GT Act as a biological entity that is viable, capable of reproduction or capable of transferring genetic material. "Gene technology" is defined as any technique for the modification of genes or other genetic material, but does not include sexual reproduction, homologous recombination or any other techniques specified in the Regulations.

As a general overview, the legislation regulates:

- biological entities that are viable, capable of reproduction or capable of transferring genetic material that have had their genes or genetic material modified by any technique aside from:

  - sexual reproduction;

  - homologous recombination; or

  - techniques described in the Regulations;

- organisms that have inherited particular traits from an organism (the initial or parent organism) where those traits occurred in the parent organism because of gene technology. This means that the legislation covers the progeny of GMOs where the progeny may have resulted from sexual reproduction but where the progeny continue to have traits that resulted from the gene technology of the initial or parent organism; and

- anything declared by the Regulations to be a GMO. This provision enables the legislation to respond to changes in technology and to be able to cover GM products (that is non- viable products of GMOs) where necessary. The capacity to declare an organism to be a GMO enables any organisms or products that are not regulated by existing regulators, such as the Therapeutic Goods Administration and the Australia New Zealand Food Authority to be regulated under the scheme. At the time of commencement of the legislation, no such things have been prescribed.

Approvals

Under the legislation, all dealings are prohibited unless they are approved. Approved dealings are subject to certain conditions and requirements.

Dealings with genetically modified organisms (GMOs) have to be approved in one of four ways. The four different types of “approvals” are exempt dealings with GMOs, notifiable low risk dealings (NLRDs), those that are included on the GMO register or are licensed by the Regulator.

Exempt dealings

These dealings with GMOs have been assessed over time as posing negligible risks. They do not require licensing and a case by case risk assessment by the Regulator. Exempt dealings must be contained within a facility and must not involve the intentional release of the GMO into the environment.

Notifiable low risk dealings (NLRDS)

These dealings with GM0s have been assessed over time as posing low risks provided certain risk management conditions are complied with. NRLDs must be notified to the Regulator, conducted within a facility certified to be at least Physical Containment Level 2, undertaken within an accredited organisation, and if transported, must be transported in accordance with guidelines issued by the regulator for the transport of GMOs. NLRDs with GMOs must not be released into the environment.

Those included on the register

Once dealings have been licensed for a certain period of time, they may be entered on the GMO register. Before they are included on the register, the Regulator is satisfied that the dealings are sufficiently safe that they cannot be undertaken by anyone, and that safety does not depend on oversight by a licence holder.

Licensed dealings

The Regulator may issue two types of licences for all dealings that are not exempt, NLRDs or included on the register. These licences are for dealings with GMOs that do not involve the intentional release of a GMO into the environment and licences for dealings with GMOs that do involve the intentional release of a GMO into the environment.

PHYSICAL CONTAINMENT LEVEL 1 (PC1 Laboratory Facility)

The work that can be conducted in a facility that is certified as a Physical Containment Level 1 (PC1) Laboratory Facility includes dealings (notifiable low risk dealings or licensed dealings) with low risk, non-infectious GMOs specifically approved by the Regulator.

In the absence of an express, written authorisation from the Regulator to conduct a particular dealing in accordance with the requirements of this containment level, dealings must only be conducted in facilities certified to at least PC2. (The particular containment level will be dependent on the specific nature of the dealings proposed to be conducted.)

These requirements do not apply to exempt dealings which, as required under subregulation 6(c) of the Regulations, must be carried out in facilities that comply with AS/NZS 2243.3:1995 (*Safety in laboratories: microbiology*) for Physical Containment Level 1. Facilities used for exempt dealings do not have to be certified by the Regulator.

Range of activities

- A PC1 facility requires no special containment equipment and is suitable for work with microorganisms in Risk Group 1. This level of facility with its practices and equipment is appropriate for student and undergraduate teaching and for food industry laboratories. A PC1 laboratory is suitable for work with microorganisms where the hazard levels are low and where laboratory personnel can be adequately protected by standard laboratory practice. The organisms used are not known to cause disease in healthy adults (that is, organisms in Risk Group 1). Work may be carried out on the open bench. Wherever possible, human blood and body fluids should not be handled in a PC1 laboratory. This also applies to so-called “Control” or “Normal” human fluid samples as the exclusion of all pathogens cannot be guaranteed.

Laboratory facilities

The facilities must comply with AS/NZS 2982,1:1997 – *Laboratory design and construction – General requirements*, and must meet the following requirements:

- Benchtops must be:
    - impervious to water,
    - resistant to reagents, solvents and disinfectants used in the laboratory, and
    - able to withstand heat generated by general laboratory procedures, such as flaming loops and heating media.
- Furniture must be ergonomically suitable for use in the laboratory. The heights of laboratory stools and chairs should be adjustable and commensurate with heights of the benches and safety cabinets. Seats should be of smooth, impervious material to facilitate cleaning.
- Washbasins with hot and cold water services must be provided in each laboratory room, preferably near the exit.
- Open spaces between and under benches, cabinets and equipment must be accessible for cleaning.
- Internal fittings and fixtures, such as lights, air ducts and utility pipes must be arranged to minimise the horizontal surface areas on which dust can settle.

Personal protective clothing and equipment

The following requirements apply to clothing and apparel worn in the laboratory:

Protective clothing, preferably in the form of a theatre or wrap-around laboratory gown, must be worn within the laboratory to protect the front part of the body.

- Closed footwear must be worn.
- Protective clothing must be removed before leaving the laboratory area and must be stored in facilities provided.,

- Safety glasses, face shields and other protective devices must be worn, where appropriate, to protect the eyes and face from splashes and other hazards. Note: Contact lenses are not a form of eye protection. Refer to AS/NZS 1336:1997 – *Recommended practices for occupational eye protection.*

Work practices

Laboratory personnel must observe the following work practices:

- Food or drink for personal consumption must not be brought into the laboratory or stored in the laboratory refrigerators. Eating, drinking, smoking, shaving and applying cosmetics are prohibited in the laboratories. Note: Hands, pens and pencils, which can become contaminated from dirty surfaces, liquids and aerosols, should be kept away from the face.
- Staff must remove laboratory gowns and thoroughly wash their hands and fingernails before moving to areas outside the laboratory, such as a canteen, refreshment room or toilet.
- Significant spills and accidents must be reported immediately to the laboratory supervisor. A written record of accidents must be prepared and maintained.
- Laboratory reagents that may be toxic must be handled with appropriate protective equipment and containment.
- Cultures must be clearly identified, dated and appropriately stored. Cultures should not be stored for long periods on the bench, but should be transferred to a dedicated storage area, such as a refrigerator or part of a cold room.
- Where work is carried out on the open bench, care must be taken to minimise the production of aerosols.
- Care must be taken to prevent the dissemination of material while flaming a wire loop, by drawing the loop gradually from the cooler to the hotter parts of the Bunsen burner flame, or by using a hooded or an ‘electric’ Bunsen burner. Disposable loops may be used as an alternative.
- Mouth pipetting must be prohibited. Rules for the correct use of pipetting devices and syringes must be followed. Blowing out residual volumes from pipettes creates aerosols; therefore it is preferable to use pipettes calibrated to deliver.
- Work benches must be decontaminated following spills, and also when work is completed.
- Reckless behaviour in the laboratory must be strictly forbidden.
- Special precautions must be taken to ensure that reading and writing materials do not become contaminated.
- Labels must not be moistened with the tongue. The use of adhesive labels is preferred.
- Long hair should be tied back as it is both a fire and a contamination hazard.
- Because airborne fungal spores can spread in a similar manner to aerosols, Petri dish cultures of fungi should be sealed with tape to prevent dispersion of spores which may be allergenic or contaminate other cultures. Where shedding of spores occurs, dedicated incubators must be allocated for specific use in fungal work.
- Facilities, separate from the work bench, should be provided for reference documents and for writing reports.
- Laboratory waste should be decontaminated prior to disposal. If suitable, decontamination may be performed with household bleach that has been appropriately diluted. Note: Formulations of household bleach usually contain about 4% w/v (40,000 ppm) available chlorine.

- Personnel who wish to transfer material between institutions are advised to pay particular attention to the various statutory requirements regarding transport of biological materials which may be regarded as infectious.
- Public access to the laboratory should be restricted to laboratory staff. Visitors or service personnel should be accompanied by a member of the laboratory staff and no children should be allowed in these areas unsupervised. Access doors must be locked when the facility is unattended.
- Dangerous goods, hazardous chemicals and sources of radiation in the laboratory should be handled according to the NSAHS Risk Management system.

PHYSICAL CONTAINMENT LEVEL 2 (PC2 Laboratory Facility)

Range of activities

A Physical Containment Level 2 facility is suitable for work with microorganisms in Risk Group 2 (see Appendix 1) and incorporates all facilities, equipment and practices for Containment Level 1. Due to the increased potential hazard, extra conditions of access, safety equipment and staff training requirements apply. This level of facility with its practices and equipment is applicable to clinical, diagnostic, industrial, teaching and other premises where work is carried out with indigenous, moderate-risk agents present in the community, and which may be associated with animal, plant or human disease of moderate severity. With good microbiological techniques, work with these agents may be carried out on the open bench, provided that the potential for producing aerosols is low. If the potential for aerosol production is high, primary containment equipment must be used.

Laboratory facilities

Laboratory facilities must comply with the requirements set out for laboratory facilities of PC1 level and the following:

- The facility must be labelled with the following adhesive signs supplied by the OGTR:
    - A Physical Containment Level 2 (PC2) sign on the outside door(s)
    - A biohazard symbol on the outside door(s)
    - A PC2 Facility Practice sign prominently displayed inside the facility
- The name and contact number of the Facility Manager or other responsible person must be listed on or near all access doors.
- Freezers, refrigerators, incubators and/or other storage units within the facility used to store GMOs must be posted with the universal biohazard symbol, available from standard laboratory suppliers. Where freezers or refrigerators are used by multiple personnel, it is recommended that the names and telephone numbers of the users are displayed on the front of the unit.
- The facility must be a fully enclosable space contained within walls, doors, windows, floors and ceiling.
- The walls, ceilings and floors of the laboratory must be smooth, easy to clean, impermeable to liquids, and resistant to commonly used reagents and disinfectants. The floors must be of non-slip finish. Surface conduits and pipes must be mounted clear of the surface to facilitate cleaning and/or decontamination. Open spaces between and under benches must be accessible for cleaning.

- Bench tops, seats and other furniture where laboratory procedures take place must be impervious to water and resistant to chemicals used to disinfect the work surfaces and equipment.
- All air supply and exhaust vents must be fitted with fly screens. Where the laboratory is mechanically ventilated, a directional air flow into the laboratory must be maintained by extracting room air. Recirculation is permitted, but not into areas outside the level PC2 facility.
- Access doors must be fitted with automatic closers and be lockable. Facility doors must be closed when laboratory procedures are in progress.
- Windows must be closed and locked. Where the laboratory is provided with opening windows, flyscreens must be fitted. Windows must remain closed while laboratory procedures are in progress unless they are fitted with intact flyscreens.
- A basin mixer, preferably of the hands free operation type, must be provided for hand washing within the facility, as near as practicable to the exit. An antiseptic hand wash dispenser must be provided at the basin mixer.
- Eye wash facilities may be required if eye protection is not worn. Plumbed eyewash stations, mounted at an easily accessible height, should be provided. If these are not available, the provision of single-use packs of sterile eye treatment fluids is recommended. Warning: There is a danger of microorganisms growing in multiple-use eye irrigation fluids. Eye wash facilities must be used and maintained as per the manufacturer's instructions.
- Protective clothing storage or coat hanging provisions must be provided within the facility, within 3 meters of the access door(s).
- The water supply to the laboratory sinks must be fitted with back-flow protection.
- Protective clothing storage or coat hanging provisions must be provided within the facility, within 3 meters of the access door(s).
- Gloves must be removed before answering the phone. Gloves and gowns must be removed before leaving the facility.
- Hands must be washed and disinfected immediately before leaving the facility or before using any dedicated facility reading/writing areas.
- If reading or writing materials are required in the facility a separate, dedicated bench must be provided.
- To facilitate decontamination, protective covers must be provided for keyboards on workbenches.
- Reading and writing material must not be used inside a biological safety cabinet.
- Containers for infectious materials and a supply of clearly labelled disinfectants for decontamination purposes must be available.

Personal protective equipment

- Protective clothing to protect the front part of the body must be worn by all persons conducting laboratory procedures in the facility and must be removed before leaving the facility. [Note: a wrap around gown is preferable to a laboratory coat]. Protective clothing must not be stored in the same locker as street clothing.
- Closed footwear must be worn. Footwear that exposes toes or heels is not suitable. Slip-on shoes that you can easily "walk out of" in the event of stepping into a sticky solution on the floor are not suitable.

- The protective equipment requirements set out for laboratory facilities of PC1 level must be fulfilled. In addition, gloves should be worn when handling blood and body fluids or infectious materials. The hands must be thoroughly washed after gloves are removed, as minute holes may allow microorganisms to enter the gloves. Gloves must not be reused and must be discarded with laboratory wastes.
- Long hair must be tied back or covered with a hair net.
- Gloves must be worn whenever there is a risk of direct contact with GM0s.
- Gloves must be worn for work undertaken in a biological safety cabinet.
- Goggles or visors must be used where appropriate to protect the eyes from contaminated or dangerous materials, or from ultraviolet light damage (refer to AS/NZ 1336 and AS/NZS 1337).

Containment equipment

- Where significant quantities of infectious aerosols are likely to be produced, a biological safety cabinet of Class 1 (refer to AS 2252.1:2002 *Biological safety cabinets (Class I) for personnel and environment protection*) or Class 11 (refer to AS 2252.2:2004 *Biological safety cabinets – laminar flow biological safety cabinets (Class II) for personnel, environment and product protection*) must be used. Provision must be made to decontaminate the biological safety cabinets, independently of the rooms, with formaldehyde gas and for the gas to be purged to atmosphere upon completion of the procedure.
- Where large volumes, or high concentrations of infectious material are used, a centrifuge fitted with either sealed rotors or safety cups must be used.
- The use of water-operated venturi vacuum pumps, although simple in operation and inexpensive, involves considerable water use and aerosol production from the water discharge. To limit water use, closed circuit units have been developed where water is recirculated through the venturi by a small electric pump. Aerosol generation from the air exhaust requires treatment by appropriate filtration.

Work practices

The work practices set out for facilities of level PC1 must be applied in addition to the following:

- All requirements for a PC2 facility must be complied with at all times, even if work being performed in the facility involves organisms that are not GMOs.
- Access to the laboratory must be limited to laboratory personnel and individuals specified by the laboratory management. Access doors must be closed when work is being carried out and locked when the facility is unattended.
- When work is in progress access to the facility must be limited to facility personnel and persons specified by the Facility Manager.
- A manual listing all procedures used in the facility (“Facility Manual”) including safety and emergency procedures, equipment operation and maintenance procedures, and a copy of these Guidelines must be available in the facility in an easily accessible and prominent position. The Facility Manual must be reviewed and updated as appropriate, but in any case at least annually. The Facility Manual must include on its cover its version number and the date when it was last reviewed.
- Persons working in the facility must have a good knowledge of the physical operation and design of the facility. They must be familiar with the protocols specific to the operation of the facility as set out in the Facility Manual.

- Operations which may generate aerosols must be done in a biological safety cabinet. The installation of and use (including fumigation) of the biological safety cabinet must comply with the requirements and recommendations contained in the latest version of AS/NZS 2647: *Biological safety cabinets - Installation and use*. Provision must be made for decontamination of the cabinet in accordance with the procedures outlined in AS/NZS 2647 or any other method specifically approved (in writing) by the Regulator. The Biological Safety Cabinet must be validated by a NATA approved technician at least every 12 months and recorded in a notice, on or adjacent to, the Biological Safety Cabinet. A period of at least 5 minutes must be allowed for any aerosols to settle after the procedure is completed and before opening homogeniser or sonicator containers in a biological safety cabinet or other approved equipment. These procedures must be reflected in the Facility Manual.
- All facility personnel must be trained in the requirements of the OGTR PC2 Laboratory Facility guidelines. Only trained personnel are to clean contaminated equipment and surfaces, or handle hazardous material.
- Facility personnel must indicate to the certification holder that they fully understand their training in the OGTR requirements by signing a record of their training after completion. A record of those trained must be kept and made available on request.
- Laboratory personnel must receive instruction and training, with regular updates, in handling pathogens.
- The Facility Manager must establish policies and procedures so that only persons who have been advised of the potential hazards associated with GMO dealings conducted in the facility and meet specific entry requirements, such as vaccination, may enter the facility.
- Records kept by the Facility Manager must include:
    - a copy of the facility instrument of certification
    - any letters of approval from the Regulator for a variation to standard procedures
    - copies of licences for dealings not involving intentional release (DNIRS) being conducted in the facility
    - copies of notices from the ISC specifying the specific notifiable low risk dealings (NLRDS) that have been notified to the Regulator and are being conducted in the facility; and
    - a list of the exempt dealings that are being conducted in that facility.
- Mouth pipetting is prohibited in the facility. This prohibition must be recorded in the Facility Manual. Procedures for the correct use of pipetting devices and syringes must also be recorded in the Facility Manual and must be followed.
- Live animals not involved in the work being performed are not permitted in the facility.
- The use of syringes and needles must be restricted to parenteral injection (that is, injection administered outside of the alimentary canal), and aspiration of fluids from laboratory animals and diaphragm-capped bottles. After use, the needle and syringe must be placed in a puncture-resistant container (refer to AS 4031:1992 – *Non-reusable containers for the collection of sharp medical items used in health care areas)* for disposal, preferably by incineration. Before disposal, needles must not be removed, bent, sheared or replaced in a sheath or guard unless the recapping/removal procedures can be carried out by a safe method with suitable equipment. Particular care must be taken when using syringes and needles, as needlestick injuries make up a large portion of laboratory accidents.
- Laboratory staff must advise maintenance and service personnel of the special microbiological hazards in the laboratory.

- Procedures for collecting specimens from patients must be closely controlled. Appropriate sterile equipment must be provided, and used, to ensure the safety of the patient and of the laboratory staff.
- All clinical specimens must be regarded as potentially hazardous. Leaking containers must be disinfected, sterilised and discarded, except where a replacement is obtainable.
- For manipulations such as shaking, mixing, and ultrasonic disruption, a biological safety cabinet (BSC) or other equipment designed to contain the aerosol must be used. A period of at least five minutes must be allowed for aerosols to settle before opening homogeniser or sonicator containers in a biological safety cabinet. Note: Large items of equipment will interfere with the airflow pattern in a Class II BSC.
- The subculture of certain Risk Group 2 organisms requires special precautions. These include eye protection or subculture in a BSC and the use of gloves and vaccinations.
- Care must be taken when carrying material likely to contain live organisms between laboratories or to autoclaves within the building. Any container of viable organisms must be transported within a second unbreakable and closed container which can be readily decontaminated.
- Potentially contaminated re-usable glassware must be autoclaved or chemically disinfected prior to washing and re-use. For chemical disinfection, pipettes must be placed in a disinfectant solution, tip-first and fully immersed, to minimise the production of aerosols. If pipettes are to be autoclaved, a detergent solution is acceptable.
- Microbial waste must be incinerated, or autoclaved before disposal. However, all waste involving genetically manipulated organisms must be steam-sterilised before disposal.
- Solid microbial waste (eg agar cultures) is to be placed in Autoclave bags provided and steam-sterilised with the bag open. Afterwards the bag is closed with a twist-tie and placed in the contaminated waste bad (Purple bag). Contaminated plasticware and used flasks can be placed directly into the purple anatomical waste bags (these are removed in sealed Sulo bins and treated by high-temperature incineration).
- Special care must be taken in handling blood, serum, other body fluids and substances that are visibly contaminated with blood, as these may contain hazardous viruses, such as hepatitis B or HIV. This risk extends to human sera and derivatives such as control reagents (both positive and negative) in diagnostic and other procedures. Note: Although existing test methods for hepatitis B virus are very sensitive, they do not entirely preclude the possibility of viral contamination. The fact that a serum sample is used as a negative control for some particular test does not necessarily mean that it is free of hepatitis viruses.
- A pest control program against insects, birds and animals should be instituted (see Attachment 2).
- The smelling and sniffing of bacterial cultures for odours is prohibited.

Decontamination

- Any unintentional release of GMOs from the laboratory must be reported to the Regulator as soon as practicable.
- Animals and plants not used in the work performed in the facility must be regarded as waste on removal from the facility and decontaminated by pressure steam sterilisation or incineration.
- All open spaces between and under benches, cabinets and equipment must be kept sufficiently unencumbered to enable these areas to be easily cleaned and/or decontaminated.

- Work benches and surfaces must be decontaminated with a chemical disinfectant treatment immediately after any spills, and when work is completed, at least daily.
- All work surfaces and equipment, in relevant areas of the facility, must be decontaminated before maintenance is carried out. Equipment used for handling GMOs or material contaminated with GM0s, must be decontaminated prior to leaving the facility by autoclaving, a chemical disinfectant treatment, or any other method specifically approved (in writing) by the Regulator.
- Where a chemical disinfectant treatment is used for decontamination, the treatment must be known to be effective for the proposed use. Liquids can be disinfected with either sodium hypochlorite or iodine provided that the final concentration of available chlorine or iodine is greater than 0.2% (ie a 1/5 dilution of Milton or Betadine stocks) and exposure time must be at least one hour before disposal. If sodium hypochlorite solutions are used, they must be prepared freshly and changed daily.
- Commercial chemical disinfectant preparations (eg Virkon S viricidal, Decon 90, Biogram) must be used at concentrations specified by the manufacturer.
- Where a chemical disinfectant treatment is used for decontamination, the treatment must be known to be effective for the proposed use.
- A supply of disinfectants for decontamination purposes must be available in the facility. The disinfectants must be clearly labelled including their concentration and date of preparation.
- The Facility Manual must contain procedures for cleaning up spills, general clean- up, contingency plans for spills on skin and other emergencies, including notification requirements and contact details in the event of the Facility Manager's absence. All facility users must follow these procedures, unless the circumstances require otherwise to ensure the safety of people and/or the environment.
- Any person involved in, or observing, a spill or any other accident or incident involving GMOs must report this to the Facility Manager who must notify the IBC. Records of such incidents should be kept by the IBC and made available if requested.

Waste Disposal

- A pressure steam steriliser must be provided where autoclaving of infectious laboratory wastes is required.
- GMOs, organisms infected with GMOs, equipment or protective clothing contaminated with GMOs, and liquid and solid wastes containing GMOs, must be decontaminated by pressure steam sterilisation (autoclaving), chemical treatment, incineration or any other method approved in writing by the Regulator. Chemical disinfectant treatment must be in accordance with Appendix E of AS/NZS 2243.3:2002. Incineration must be in a high temperature, high efficiency, EPA-approved incineration facility. Protective clothing that has not been contaminated with GMOs may be washed using normal laundry methods.
- Where an autoclave is used for decontamination:
  - The facility must contain an autoclave or have an autoclave located within the same building as the facility that is accessible to facility users.
  - Provision must be made to allow for the penetration of steam into the container during autoclaving.
  - The coldest part of the autoclave must be exposed to a minimum temperature of 121^{o}C for at least 15 minutes (or equivalent temperature/time).
  - Autoclave tape or similar indicators must be used to differentiate loads that have been processed from those that have not.

- The temperature of each cycle must be monitored by one of the following means: a thermocouple and recorder; a maximum thermometer; a chemical indicator; spore strips; or readings from the autoclave panel.
- The effectiveness of the decontamination by the autoclave must be tested with biological indicators once every 10 autoclave cycles or at least monthly, and the results posted on or adjacent to the autoclave.
- The operation of the autoclave must be validated by a NATA approved technician at least every 12 months and recorded in a notice, on or adjacent to, the autoclave.

Transport

- All GMOs, and waste potentially contaminated with GMOs, being transported out of the facility (including to decontamination or disposal facilities) must be transported in accordance with the "Guidelines for the transport of GMOs" (see Appendix 2). Gloves must be worn while transferring primary containers between the storage unit and the secondary container used for transport. Any spills that occur during storage outside the facility or when transferring to the storage unit, must be reported to the Regulator as soon as practicable. The spilt material and the area must be decontaminated.
- Transport of GM animals must be undertaken in accordance with the following requirements:
  - the animals must be contained in a manner which will prevent the animals from escaping;
  - a person with experience in handling the particular type of animal must take delivery of the animals.
  - Accounting procedures must be in place to ensure that all animals sent are delivered.

Storage

All cultures of fungi and other spore-dispersing organisms must be sealed during storage to prevent dispersal of spores. All cultures must be clearly marked as to their contents and the date of inoculation.

- If GMOs or organisms infected with GMOs are to be stored in a cold storage facility (ie a freezer, refrigerator, cold room or freezer room), outside of the facility, the cold storage facility must be:
  1. locked at all times, except when being accessed to insert or retrieve material;
  2. posted with the universal biohazard symbol, available from standard laboratory suppliers; and
  3. maintained as per manufacturer's instructions, and records of any equipment malfunctions and repair activities (including decontamination of any leaking materials) must be recorded and made available if requested.
- GMOs or organisms infected with GMOs being stored within cold storage facilities outside of the facility, must be doubly contained in a sealed primary container, within a sealed secondary unbreakable container. In the case of a small storage unit such as a fridge, freezer or liquid nitrogen container, the secondary storage container may be the storage unit.
- A record is to be maintained of the location of all stored GMOs which is to be sent to the Facility Manager for collation in a central database.

'Safe storage' means that all of the following are demonstrated:

1. *All the GMOs are stored in sealed containers; AND*
2. *All the sealed containers holding the GMOs are labelled in a way that enables details about the GMOs to be identified; AND*
3. *All the GMOs are stored inside a facility that is currently certified by the* OGTR to a minimum of PC2 level, OR
4. *All the GMOs are stored in a storage* ***unit that completely contains the GMO under conditions which limit the ability of the GMO to propagate*** *and which is either:*
    - *Lockable; or*
    - *Located in a secure area that prevents access to the cabinet from persons not responsible for its contents.*

HANDLING BLOOD AND OTHER PRODUCTS OF HUMAN ORIGIN

Management of exposures

If a worker has a parenteral exposure, for example needle-stick or a cut, or mucous membrane exposure, to blood or other body fluids, the following procedures should be implemented:

- Treat the puncture wound or cut by liberal washing with soap and water and/or dilute hypochlorite solution.
- If the face is splashed with blood, rinse the, eyes and mouth (which present exposed membranes) gently with water to minimise the risk of infection.
- Ensure that the incident is reported to the laboratory supervisor, that any incident/accident documentation has been completed, and that medical attention is sought.
- Consult with OHS Nurse for follow up in normal working hours or approach Accident and Emergency out of hours as per NSH protocol.
- If possible, identify the source material and test for the presence of HIV or hepatitis B.

If the source material tests positive or is unknown:

- The worker should be counselled regarding the risk of infection and should be clinically and serologically evaluated.
- For suspected HIV exposure, immediate commencement of AZT therapy should be considered by the treating medical officer and the worker should be retested for seroconversion in three months and, if negative, again in six months' time.
- For suspected hepatitis B exposure: if less than five years has elapsed since an effective vaccination, no further action need be taken. If the person exposed did not respond effectively to the vaccine or has never been vaccinated, then the treating medical officer should consider HBIG and/or commencing a course of vaccination.

If the source material tests negative, no further action need be taken unless considered necessary by the treating medical officer. A course of hepatitis B vaccinations should be commenced if the worker has not been vaccinated previously.

PHYSICAL CONTAINMENT LEVEL 2 (PC2 Animal Containment Facility)

General

The work that can be conducted in a facility that is certified as a Physical Containment Level 2 (PC2) Animal Containment Facility includes work with GM animals, and/or animals containing GMOs, that present a low to moderate potential risk to people and/or the environment.

Facilities

1. The facility must be labelled with the following adhesive signs as supplied by the OGTR:
    a. a Physical Containment Level 2 (PC2) sign on the outside of the facility door or the anteroom door;
    b. a biohazard symbol on the outside of facility access door or the anteroom door; and
    c. a PC2 Facility Practice sign prominently displayed inside the facility.
2. The facility must be a fully enclosable space contained within walls, doors, windows, floors and ceilings.
3. The facility must have an anteroom. Entry to the facility must be through the anteroom. Emergency exits must not be used except in emergencies. Facility doors and doorways must be designed to prevent the escape of the animals contained within the facility.
4. Walls, floors, ceilings and benches must be smooth, impermeable to water, cleanable, and resistant to the cleaning agents and/or disinfectants used in the facility. Facility furniture, including seating, must be washable.
5. Any openings in the walls, ceiling or roof, such as air vents, must be screened with rodent proof mesh. Where a dealing being conducted in the facility involves animals infected with an agent capable of being transmitted by arthropods, then strategies must be in place to prevent the arthropods from entering or leaving the facility.
6. If the facility has drainage exits, they must be fitted with barriers (e.g. floor wastes or mesh) to prevent rodents or any other animal from entering the facility via the drains and to prevent the escape of animals from the facility. Where a dealing being conducted in the facility involves animals infected with an agent capable of being transmitted by arthropods, the drains must also be screened or designed to prevent arthropods from entering or leaving the facility via the drains (e.g. by use of "s" bends so the drain is permanently filled with water).
7. The joints between structural components of the facility must be sealed.
8. A wash basin must be provided for hand washing within the facility. The wash basin must be fitted with a basin mixer of the hands-free operation type.
9. Eyewash facilities (either a plumbed eyewash facility or single-use packs of sterile eye irrigation fluids) must be provided within the facility. Eyewash facilities must be used and maintained in accordance with the manufacturer's instructions.
10. The facility must contain a pressure steam steriliser (autoclave) or have an autoclave that is accessible to facility users. If the autoclave is not located in the facility, it is preferable that it be located within the same building as the facility.
11. Designated storage or hanging provisions for protective clothing must be available within the facility or the anteroom.

12. A supply of disinfectants for decontamination purposes must be available in the facility. The disinfectants must be clearly labelled with the contents and, where necessary, the expiry date.
13. Open spaces between and under benches, cabinets and equipment must be accessible for cleaning.

Personal protective clothing and equipment

1. Protective clothing to protect the front part of the body must be worn by all persons performing procedures in the facility.
2. Closed footwear must be worn.
3. Protective clothing must be removed before leaving the facility. This may occur in the anteroom.

Containment equipment

1. If procedures that generate aerosols containing GMOs are to be performed in the facility, the facility must contain a biological safety cabinet, or other equipment specifically approved in writing by the Regulator that is designed to contain aerosols.
2. Installation, use and decontamination of the biological safety cabinet must be in accordance with the requirements of AS/NZS 2647: "*Biological safety cabinets - Installation and use*".
3. The biological safety cabinet must be tested at least every 12 months by a NATA accredited organisation. The cabinet must be labelled to show its test status.

Work practices

1. All requirements for a PC2 Animal Containment Facility specified in the Certification Instrument issued by the Regulator must be complied with at all times, even if the work being performed in the facility involves organisms that are not GMOs.
2. Access to the facility must be restricted to authorised persons and/or authorised classes of persons.
3. Facility doors must be closed while work is being undertaken in the facility and must remain locked when the animals are not under supervision.
4. Windows must be closed and locked while GM animals or animals containing GMOs are in the facility.
5. All facility personnel must be trained in the requirements of the OGTR PC2 Animal Containment Facility Guidelines. Only trained personnel are to clean contaminated equipment and surfaces, or handle hazardous material.
6. Facility personnel must indicate to the certification holder that they fully understand their training in the OGTR requirements by signing a record of their training after completion. A record of those trained must be kept and made available if requested.
7. Any procedures that generate aerosols containing GMOs must be performed in a biological safety cabinet or other equipment designed to contain aerosols specifically approved in writing by the Regulator. Bedding material and waste from infected animal cages or pens must be handled in a manner that minimises the creation of aerosols.
8. Any unintentional release of GMOs from the facility must be reported to the Regulator as soon as practicable.

9. Work benches, surfaces and equipment where procedures have taken place must be decontaminated immediately after any spills containing viable GMOs and when procedures using GMOs are completed.

10. All work surfaces and equipment, in relevant areas of the facility, must be decontaminated before maintenance is carried out.

11. Equipment or protective clothing, pens, cages, bedding or wastes contaminated with GM micro-organisms must be decontaminated by pressure steam sterilisation (autoclaving), chemical treatment, incineration or any other method approved in writing by the Regulator. Chemical disinfectant treatment must be in accord with Appendix E of AS/NZS 2243.3:2002. Incineration must be in a high temperature, high efficiency, EPA-approved incineration facility. Protective clothing that has not been contaminated with GM micro-organisms may be washed using normal laundry methods.

12. Carcasses of animals infected with GM micro-organisms or GM animals infected with infectious agents must be decontaminated by pressure steam sterilisation (autoclaving), incineration or any other method approved in writing by the Regulator.

13. Where a pressure steam steriliser (autoclave) is used for decontamination:

    a. Provision must be made to allow for the penetration of steam into the container during autoclaving.

    b. The coldest part of the load must be exposed to a minimum temperature of 121 C for at least 15 minutes.

    c. Measures must be taken to ensure that loads that have been processed can be differentiated from loads that have not (e.g. autoclave tape).

    d. The temperature of each cycle must be monitored by use of one of the following means: a thermocouple and recorder; a maximum thermometer; a chemical indicator; spore strips; or readings from the autoclave panel.

    e. The effectiveness of decontamination by the pressure steam steriliser (autoclave) used by the facility must be tested monthly with biological indicators. A notice must be posted on, or adjacent to, the autoclave indicating the result and the date of the latest test.

14. All GMOs, and waste potentially contaminated with GMOs, being transported out of the facility must be transported in accordance with the "*Guidelines for the Transport of GMOs*".

15. Animals and plants not used in the work being performed in the facility, that are potentially infected with infectious agents, must be regarded as waste on removal from the facility and decontaminated by pressure steam sterilisation (autoclaving), incineration, or any other method approved in writing by the Regulator.

16. Viable animals must not be removed from the facility unless they are to be transported to a containment facility certified by the Regulator to equivalent or higher containment level. Animals must be transported in accordance with the "*Guidelines for the Transport of GMOs*".

17. All animal cages or containers must be labelled to enable identification of the animals being contained and to indicate the number of animals in the containers.

18. Large animals must be clearly marked so they can be readily identified (e.g. with a tattoo, permanent tag, microchip or permanent brand).

19. Eating, drinking, smoking, shaving and applying cosmetics are prohibited in the facility. Food or drink intended for human consumption must not be brought into or stored in the facility.

20. Long hair must be tied back or covered with a hair net, to avoid contamination, when the work of the facility involves animals inoculated with infectious agents.

21. Cuts and abrasions on the skin of facility personnel must be covered while working in the facility.
22. Only reading/writing material and computers essential to procedures performed within the facility are permitted on work benches where procedures are performed. Reading and writing material must not be used inside a biological safety cabinet. Where possible, dedicated reading/writing areas should be provided and used.
23. Persons who have been performing procedures in the facility must wash or decontaminate their hands immediately before leaving the facility or before using any dedicated facility reading/writing areas.
24. The facility and equipment in the facility must be maintained so that the facility meets the containment requirements of these Guidelines.
25. Strategies must be in place to ensure that the facility is free of pests. A record of the program and dates of specific activities must be kept and made available if requested.

EMERGENCY PROCEDURES AND SPILLS

Biological spills

Each spill of micro-organism culture should be assessed as to the risk level of the micro-organism, the amount and concentration of the spill and whether the spill has occurred within containment equipment such as a biosafety cabinet. In general, for spills occurring outside a Biosafety cabinet which contain over 10ml of viable pathogens, the routine to follow when responding to a spill is:

- Minimise the chance of inhaling the aerosol and vacate the laboratory.
- Shut the laboratory door and place appropriate signs to warn other people of the hazard.
- Remove any contaminated clothing including laboratory coat and gloves and place them in a biohazard bag.
- Wash face and hands; put on clean personal protective equipment.
- Do not allow anyone to re-enter the area for at least 30 minutes so that the aerosol has a chance to settle.
- The clean-up team should then proceed to:
  - Assess the area and degree of the spill and subsequent potential contamination.
  - Pour an appropriate disinfectant (in most cases, hypochlorite or iodophor solutions are suitable) around the spill area so that it mixes slowly with the contaminated material. Do not pour the disinfectant straight onto the spill as this procedure can result in the generation of more aerosol.
  - Gently place paper or cloth towels that have been saturated with disinfectant over the spill. Allow at least-30 minutes for the disinfectant to take effect.
  - Wipe over with disinfectant any surfaces which may have become contaminated.
  - Carefully absorb the spill and disinfectant solution into dry towels and place all contaminated material into appropriate receptacles for subsequent disposal. Do not autoclave any materials which have been soaked with hypochlorite solution due to the risk of toxic gas being produced.
  - Remove and autoclave protective clothing worn during the clean-up.

Disinfection and decontamination methods

The following chemicals are known to be effective in killing HIV and hepatitis B if used in accordance with the guidelines and manufacturers' instructions. Some disinfectants may be more appropriate than others for particular uses. A material safety data sheet should be available for all chemical disinfectants to be used. Ensure that the recommended safety precautions are followed and the correct protective equipment is worn when mixing and using disinfectants.

Sodium hypochlorite

Sodium hypochlorite has been recommended as a safe and effective agent against HIV and hepatitis B.

Use 0.5% (5,000 ppm available chlorine) for application to areas that have been grossly soiled with blood or body fluids. Porous surfaces, benches, floors, walls and other inanimate objects

likely to be contaminated but not visibly soiled should be cleaned with 0.05% (500 ppm available chlorine) sodium hypochlorite.

The hypochlorite solution should be in contact for at least 10 minutes. Spills can be wiped up with a wad of cottonwool soaked in 0.5% hypochlorite solution. Discard the contaminated cottonwool into a wastebag. It may be necessary to wipe the area again with 0.5% hypochlorite, then clean the area with water and commercial detergent.

Household bleaches contain hypochlorite and are suitable for use as a disinfectant for HIV. They should be diluted to give the appropriate concentration of hypochlorite. Gloves and eye protection should be worn when mixing bleach. Bleaches containing 1 % hypochlorite should be diluted 20-fold, that is, one part of bleach should be added to 19 parts of water. Stronger bleaches should be diluted proportionately more. This dilution will provide a 0.05% solution, which is suitable for disinfection of surfaces not directly involved in a spill of contaminated fluid. Concentrations of hypochlorites vary between brands and products of bleaches and should therefore be checked. Fresh hypochlorite solution should be prepared daily. It is important to ensure that the granular forms are dissolved completely.

Note:

- Hypochlorite solutions may be corrosive for metal objects.
- Persons using hypochlorite should wear gloves.
- All centralised disinfection services should be well ventilated.

Glutaraldehyde

Best practice is to eliminate the use of glutaraldehyde from the workplace. If need arises for the utilisation of glutaraldehyde consult with Supervisor and inform Occupational Health of usage. The following are some supportive guidelines for the usage of glutaraldehyde in the workplace.

Use 2% (w/v freshly prepared in water) in contact for 20 minutes. Fresh stocks should be used within 24 hours of activation or in accordance with manufacturer's instructions. Alternatively, approved instrument disinfectant solutions containing 1% glutaraldehyde in a stable form are widely used and are also appropriate.

- The area of use should be ventilated continuously with a mechanical exhausting system (threshold limit of 0.2 ppm).
- Users must wear gloves to minimise skin sensitisation. Double gloving is recommended if latex gloves are used.
- The use of protective clothing (such as impervious aprons) is recommended.
- Goggles should be worn to prevent splashing of the eyes.
- Glutaraldehyde should not be used as a surface disinfectant due to its toxicity.

In 1991 Worksafe Australia issued a hazard alert notice for glutaraldehyde. Consider the use of alternative means of disinfection. If this is not feasible, ensure that the material safety data sheet is available and understood by all workers using glutaraldehyde and that the correct precautions have been taken.

Ethanol

Ethanol is used as a disinfectant as a 70% (v/v in water) solution and should be in contact with the material being disinfected for at least 20 minutes. Industrial methylated spirits (which is 95% ethanol), suitably diluted, is an acceptable alternative.

Lower concentrations of ethanol have been shown to be effective in inactivating HIV, but a 70% solution is effective for a wide range of other pathogens though it cannot be relied upon to inactivate hepatitis nonA-nonB agents.

Ethanol is suitable for use on porous surfaces because it is volatile, but owing to its volatility, repeated applications may be necessary to ensure adequate contact. Use ethanol with caution near electrical appliances and naked flames because of its flammability.

Iodine

Use 0.5% iodine in 70% (v/v) ethanol in contact for at least 20 minutes. Iodophors are effective if diluted in accordance with manufacturer's instructions to give at least 100rng available iodine per litre.

Formalin

Formalin is a 37% solution of formaldehyde in water. Free HIV in suspension is inactivated by exposure to 0.5% formalin for 10 minutes. Tissues such as biopsy and autopsy specimens are penetrated very slowly by formalin - it may take several days to reach the centre of a tissue block. In histopathology, it is usually used in a concentration of 10%, which should be adequate to destroy HIV, provided the formalin has penetrated the tissue completely.

OTHER RESOURCES AND REFERENCES

Other Manuals

- NSAHS OH&S Policy and Procedures Manual
- NSAHS Radiation Safety Manual
- CCH Laboratory Safety Manual (in lieu of the RNSH Laboratory Safety Manual currently in preparation)

Other Resources

- The Office of the Gene Technology Regulator (OGTR): http://www.ogtr.gov.au
- Guidelines for Certification of PC2 Facilities / Physical Containment 2 Requirements, Version 2.2 – 7 August 2003 (OGTR Web Site).
- HSRMU- Health Services and Risk Management Unit – Northern Sydney Health http://intranet01.nsahs.nsw.gov.au/intranet/area/areahumanresources/index.htm
- Copies of Australian Standards referred to in this manual are held in the Douglas Piper Library: http://icu-web.org/icuweb/servlet/icuweb/?site_id=Library
- NSW Department of Health Policy Manuals: http://10.192.128.9/audit/internal/

Emergency Phone Numbers:

All emergencies:	33 (internal)	All emergencies:	0 000 (external)
Security:	67190	Fire Safety:	66836
Facilities:	67219	Casualty Dept.	66000

Other Phone Numbers:

DOSH:	68511
OGTR:	1800 181030

APPENDIX 1 MICROBIAL RISK GROUPS

All microorganisms potential pathogens

AS/NZS 2243.3:1995 *Safety in Laboratories - Microbiology* details safe working practices, risk groupings and physical containment levels for laboratory work involving microorganisms. The standard classifies microorganisms into risk groups and sets out physical containment levels corresponding to each risk group. Requirements are provided for laboratory facilities, personal protective equipment and work practices relating to each physical containment level. The safest procedure to adopt while working with microorganisms is to regard all microorganisms as potential pathogens, and treat them accordingly. Thorough knowledge and use of good laboratory practice are of the utmost importance in the safe handling of infectious material. Certain microorganisms are hazardous if they are handled on the open bench, usually because they are readily transmitted by aerosols, or their infectious dose is small. Others present special hazards from risk of accidental self-inoculation. Special containment equipment and laboratory designs have been developed for the safe handling of hazardous organisms.

Classification of infective microorganisms by risk group

The following classification is based on the pathogenicity of the agent, the mode of transmission and host range of the agent, the availability of transmission and host range of the agent, the availability of effective preventive measures and the availability of effective treatment:

Biological safety Risk groupings of infectious microorganisms

- Risk Group 1 (low individual and community risk). This is a microorganism which is unlikely to cause human, plant or animal disease.
- Risk Group 2 (moderate individual risk, limited community risk). This is a pathogen that can cause human, plant or animal disease, but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause infection, but effective treatment and preventive measures are readily available and the risk of spread of the infection is limited.
- Risk Group 3 (high individual risk, limited community risk). This is a pathogen that usually causes serious human or animal disease and may present a serious hazard to laboratory workers. It could present a risk if spread in the community, but there are usually effective preventive measures or treatment available.
- Risk Group 4 (high individual and community risk). This is a pathogen that usually produces life-threatening human or animal disease. It represents a serious hazard to laboratory workers and is readily transmittable from one individual to another. Effective treatments and preventive measures are not usually available.

Risk-grouping of microorganisms by type

This section outlines the risk groups into which different types of microorganisms are categorised. For an extensive list of examples of microorganisms belonging to Risk Groups 2, 3 and 4, refer to Tables 4.1 to 4.11 in ASINZS 2243.3:1995. The standard does not provide a table for Risk Group 1, as the number of relevant microorganisms is large. To assist those wishing to use safe microorganisms for student work, typical examples are microorganisms living in soil, such as *Azotobacter* sp; the vinegar-producing microorganism, *Acetobacter* sp; and brewer's and baker's yeast. Some microorganisms that are regarded as part of the normal flora of humans or animals may be pathogenic for immuno-compromised individuals.

Bacteria, Chlamydiae, Rickettsiae and Mycoplasmas

These and all other “pathogenic” microorganisms are Risk Group 2 unless proved otherwise.

Parasites

Many parasites are regarded as Risk Group 2, with respect to their infective stages. Preparations that are known to be free of infectious stages may not require a containment level corresponding to this risk group.

Fungi

Risk Groups 2 and 3 are restricted to fungi that may pose a hazard for healthy persons. Fungi that infect following traumatic inoculation, for example, *Phialophora verrucosa* and *Pseudoallescheria boydii*, together with a large number of fungi normally saprophytic but which cause infections in the compromised host, are excluded and are classified in Risk Group 1. Unless otherwise stated, fungi from clinical specimens should be handled at Physical Containment Level 2.

Viruses

Tables 4.7 and 4.8 in AS/NZS 2243.3:1995 list examples of viruses for Risk Groups 2 and 3. Risk Group 2 viruses include hepatitis B, Dengue Fever, Ross River virus, Yellow Fever (Strain 17D). Risk Group 3 viruses include Eastern and Western Equine Encephalitis, Japanese Encephalitis, Yellow Fever and Lentivirinae - Human Immunodeficiency Virus. Table 4.9 of the standard lists examples of viruses of Risk Group 4; these include Ebola virus, Russian spring summer encephalitis and Lassa virus.

Exotic animal viruses

Tables 4. 1 0 and 4. 1 1 of the standard which set out lists of animal viruses which are exotic to Australia. Table 4.10 also includes some viruses that are potentially infectious to humans. Viruses in both of these lists must be handled only at the CSIRO Australian Animal Health Laboratory.

Fish pathogens

Fish pathogens should be handled using good microbiological practices to avoid contamination of the environment. Some bacterial species implicated in diseases in fish are also known to cause disease in humans, for example, *Aeromonas hydrophilia*.

Plant pathogens

Plant pathogens do not cause disease in humans or animals, but should be handled with good microbiological practices to avoid liberation into the environment. Anybody using plants in genetic manipulation work should consult the Genetic Manipulation Advisory Committee (GMAC) Guidelines for Small Scale Genetic Manipulation Work. For work, with plant viruses of Australian origin, refer to Viruses of plants in, Australia.

APPENDIX 2 GUIDELINES FOR THE TRANSPORT OF GMOS

What does "transport" a GMO mean?

The GT Act regulates all "dealings" with GMOs. One of the "dealings" with GMOs that is provided for in the Act is the transport of GMOs. "Transport" is not defined in the GT Act and takes its ordinary meaning. Within these Guidelines, it is taken to mean "to carry or convey from one place to another" and includes:

- all movements of a GMO from a certified facility to any location outside the certified facility. For example:
    - movement from one certified facility to another certified facility;
    - movement from the certified facility to another area of the building that has not been certified by the Regulator; and
    - movement of the GMO from the certified facility to a place where the GMO is destroyed or disposed of (for example, to an autoclave).
- all movements from a location specified in a license (such as field trial site) to another location.

The Guidelines do not apply to transport:

- from a place in a certified facility to another place within the same certified facility (for example, from one side of a PC2 laboratory to the other); or
- from a place in a location specified in a licence to another place within the same specific location (for example, from one part of a field trial site to another part of the same site).

When can my organisation transport a GMO and how does my organisation get approval to transport a GMO?

In summary, the GT Act provides that a person must not *deal with* a GMO, including *transport* a GMO, unless the dealing with the GMO is:

- authorised by a GMO licence (including a "deemed licence" issued as a "GMAC advice to proceed");
- a notifiable low risk dealing;
- an exempt dealing; or
- on the Register of GMOs.

In order to decide what type of GMO you are dealing with, and therefore what type of approval you will need from the Regulator in order to transport the GMO, you will need to examine the legislation. The *Handbook on the Regulation of Gene Technology In Australia* may also be a useful resource. It explains in simple terms, and with examples, the types of GMOs that fall into each category.

The GT Regulations include Schedules which detail all of the "exempt dealings" with GMOs and also all of the "notifiable low risk dealings" with GMOs. All other dealings with GMOs MUST be licensed by the Regulator.

Exempt dealings with GMOs

If the GMO that you are intending to transport is on the list of **exempt dealings** – you do not need any approval from the Regulator to transport the exempt GMO. Exempt dealings must not, however, involve an intentional release of the GMO into the environment and must be conducted in accordance with Australian Standard AS/NZS 2243.3:1995 *(Safety in laboratories: microbiology*) for physical containment Level 1.

Notifiable low risk dealings with GMOs

If the GMO that you are intending to transport is on the list of **notifiable low risk dealings,** you must notify the Regulator that you are intending to deal with the GMO (which may include transporting the GMO). If the GMO is on the list of NLRDs, all dealings with the GMO (including transport) may be conducted provided the conditions in the Regulations are complied with. This includes the conditions contained in these Guidelines for the transport of the GMO. The process for notifying the Regulator (and the other requirements for conducting NLRDs) are included in the Gene Technology Regulations 2001 and the *Handbook on the Regulation of Gene Technology in Australia.*

Dealings with GMOs on the GMO Register

If the GMO that you are intending to deal with is on the **GMO Register**, you may deal with the GMO provided that you comply with any conditions that are noted on the GMO Register. If there is a condition attaching to the GMO that is on the GMO Register, that requires compliance with these Guidelines, then these Guidelines must be complied with in relation to any transport of the GMO. At the commencement of the legislation, there are no dealings with GMOs on the GMO Register.

All other dealings with GMOs

If the GMO is not on the list of exemptions, not on the list of notifiable low risk dealings and not on the GMO Register – you must not transport the GMO unless you have a licence from the Regulator or are covered by a licence from the Regulator that allows you to transport the GMO. The *Handbook on the Regulation of Gene Technology in Australia* provides information about how to go about applying for a licence from the Regulator.

What are the conditions that apply to the transport of GMOs?

The transport of any GMOs that are on the list of **NLRDs** must be undertaken in accordance with the conditions for transport set out in Chapter 2 of these Guidelines. In relation to **licensed dealings with GMOs**, the Regulator may apply whatever conditions are necessary in order to manage any risks posed by the GMO (including by transport of the GMO). The Regulator may:

- require that any transport of the GMO be conducted only in accordance with the conditions for the transport of GMOs detailed in these Guidelines; or
- impose additional conditions if he/she consider it necessary to manage the risks posed by the GMO;
- impose lesser conditions in relation to the transport of GMOs if the conditions prescribed in these Guidelines are not necessary to be complied with, given the negligible risk posed by the GMO. For example, in the case of GMOs approved for intentional release into the environment where such approval is for the commercial release of the GMO (rather than for field trials), the Regulator may apply lesser conditions relating to transport.

In relation to licensed dealings with GMOs, you must consult the individual licence to check what conditions apply for transport of the GMO. If the licence provides that these Guidelines must be complied with, then any person transporting the GMO described in the licence must comply with the conditions detailed in Chapter 2 of these Guidelines.

Who must comply with the conditions for transport of GMOs set out in these Guidelines?

In summary, the following people must comply with the conditions for transport of GMOs contained in these Guidelines:

- anyone undertaking a NLRD with a GMO;

- in relation to a licence that specifically provides that these Guidelines must be complied with:
    - the holder of the licence;
    - and anyone covered by the licence;
- in relation to an Advice to Proceed issued by GMAC that specifically provides that these Guidelines must be complied with:
    - the holder of the Advice to Proceed; and
    - anyone covered by the Advice to Proceed.

For the purposes of the Act, there is no practical difference between a licence and a GMAC Advice to Proceed. The concept of a GMAC Advice to Proceed was developed to minimise disruptions flowing from the commencement of the new regulatory system for GMOs. A GMAC Advice to Proceed is a "deemed" license that operates for up to two years.

All organisations currently undertaking dealings with GMOs have been issued with a GMAC Advice to Proceed in respect of their dealings with GMOs. From 21 June 2001, the holder of the GMAC Advice to Proceed will effectively be a licence holder under the Act and the holder of the GMAC Advice to Proceed (and any persons covered by the GMAC Advice to Proceed) must comply with the Advice to Proceed as if it were a licence under the Act. The conditions set out in the GMAC Advice to Proceed may include a condition that any applicable technical and/or procedural guidelines issued by the Regulator, including the Guidelines for the Transport of GMOs, must be complied with by people dealing with the GMO. If a condition to this effect appears in the GMAC Advice to Proceed then these Guidelines must be complied with in relation to the transport of the GMO.

IMPORTANT NOTE
If a licence issued by the Regulator (or an Advice to Proceed issued by GMAC) provides that transport of the GMO must be in accordance with the conditions contained in these Guidelines, then it will be the responsibility of the licence holder (or holder of the GMAC Advice to Proceed) to make sure that people transporting the GMO are:

- covered by the licence; and
- aware of the relevant conditions for transport of GMOs contained in these Guidelines; and
- if necessary, have been properly trained to enable them to comply with the conditions of transport.

Conditions relating to informing people of conditions of transport of GMOs

- An Accredited Organisation or person that is:
    - the holder of a GMAC Advice to Proceed;
    - authorised to undertake notifiable low risk dealings; or
    - the holder of a licence issued by the Regulator;

  must take all reasonable steps to ensure that anyone authorised by the Organisation to transport the GMOs, is aware of any conditions of transport that must be complied with.
- The Accredited Organisation must have put internal procedures in place to ensure that anyone transporting the GMOs is made aware of the conditions relating to the transport of GMOs.

Conditions relating to transport of GM micro-organisms

- Transport of GM micro-organisms must be undertaken in accordance with the following requirements:
    - the GM micro-organism must be wholly contained within a primary sealed container; and

- the primary sealed container must be packed in a secondary sealed unbreakable container.

- The secondary unbreakable container must be labeled to indicate that it contains GM microorganisms, and the label must include the telephone number of a person to contact should the package be damaged or lost.
- Accounting procedures must be in place to ensure that the same number of containers sent is delivered.
- Following transport of the GMO, the primary sealed container and the secondary unbreakable container must be decontaminated, by whatever means necessary, to ensure that no residual GMO is retained, or the containers must be destroyed.

Guidance Notes:

- A 'primary sealed container' is a container that is designed to wholly contain the GMO. For example, a sealed plastic tube or a petri dish sealed with parafilm.
- A secondary unbreakable container is an airtight container that is designed to hold the primary sealed container and to ensure that, should the primary sealed container break during transport, the GMO cannot escape the secondary unbreakable container. An example of a secondary unbreakable container is a sealed airtight plastic container such as a tupperware container.
- It is important that the containers used are appropriate for containing the particular GMO bearing in mind that the purpose of the condition is to ensure that there is no accidental escape of the GMO during transport.
- These conditions must be complied with in any circumstances where a person wishes to move the GMO from a certified facility. Examples are carrying a GMO to the autoclave or other disposal site, or sending it from one certified laboratory to another certified laboratory.
- For example, one laboratory within a building may be certified by the Regulator as an area in which work with GMOs may be conducted. If a person wishes to move the GMO outside that facility, even if it is still within the building, they must comply with these conditions, particularly the requirement that the GMO must be contained in a primary sealed container, within a secondary unbreakable container.

Conditions relating to transport of GM animals (excluding GM insects and GM aquatic organisms)

- Transport of GM animals must be undertaken in accordance with the following requirements:
    - the animals must be contained in a manner which will prevent the animals from escaping;
    - a person with experience in handling the particular type of animal must take delivery of the animals.
- Accounting procedures must be in place to ensure that all animals sent are delivered.

Guidance Notes:

- With regard to the transport arrangements for GM animals, two principles are paramount:

➢ the need to prevent the animals from escaping, especially with regard to reasonable contingencies such as accidents *en route*, so that they will not interbreed with feral populations; and

➢ the need to ensure that the animals are properly identified and duly arrive at the intended destination.

- It is expected that the person taking delivery of the animals would be a competent biologist with experience in handling animals of the type transported.
- It is expected that the Organisation will develop all necessary internal procedures and protocols to ensure that these conditions are met.
- The Director of the Animal Resources Centre may be contacted for the purchase of animal boxes approved by the airlines for the transport of specific pathogen-free animals by air. These may be adapted for specific needs. The contact details for the Director of the Animal Resources Centre are as follows:

  The Director

  Animal Resources Centre

  PO Box 1180

  CANNINGVALE WA 6155

  Ph: 08 9332 5033

  - It should be noted that the requirements detailed above relate only to transport of animals that are GMOs as distinct from microorganisms (for example GM bacteria or viruses) that may be proposed to be used in animals. The transport of such micro-organisms must be in accordance with the requirements for transport of microorganisms. If the animals contain GM micro-organisms additional precautions may need to be taken and advice from the Regulator should be sought.

Conditions relating to transport of GM insects, including live insects and insect cell cultures infected with genetically modified pathogens

- Transport of GM insects must be undertaken in accordance with the following requirements:
  - the insects must be contained in a holding container, adequately sealed to prevent the escape of insects;
  - the holding container must be placed in another, wholly sealed unbreakable (outer) container for transport.
- The wholly sealed unbreakable (outer) container must be labelled to indicate that it contains GM insects and/or their pathogens, and the label must include the telephone number of a person to contact should the container be damaged or lost.
- Accounting procedures must be in place to ensure that the same number of containers sent is delivered.
- The transport containers must be decontaminated by autoclaving following transfer of the transported insects into new containers.

Guidance Notes:

- As detailed above, the insects must be contained in a holding container adequately sealed to prevent the escape of insects. This inner container is not required to be completely sealed (air-tight) as it is recognised that the container must be able to allow oxygen to reach the insects contained within. An example of an appropriate inner container would be a tube with a cotton wool stopper.
- This inner container (the holding container) must however be placed in another wholly sealed unbreakabale container, such as a tupperware container. This secondary container must be unbreakable (to ensure that even if there is damage to the container during transport, the insects cannot escape) and must be sealed to ensure that should the insects escape the inner holding container, they cannot escape the outer container.

- It is important that the containers used are appropriate for containing the particular GMO bearing in mind that the purpose of the condition is to ensure that there is no accidental escape of the GMO during transport.
- These conditions must be complied with in any circumstances where a person wishes to move the GMO from a certified facility. Examples are carrying a GMO to the autoclave or other disposal site, or sending it from one certified laboratory to another certified laboratory.

Conditions relating to compliance with relevant packaging and transport regulations

All relevant packaging and transport regulations must be complied with for the transport of GMOs including, where relevant:

- the International Air Transportation Association (IATA), *Dangerous Goods Regulations*;
- the Australia Post *Dangerous Goods and Packaging Guide*;
- the *Australian Code for the Transport of Dangerous Goods by Road and Rail*.
- local public health regulations (for the transport of infectious wastes).

Guidance Notes:

The IATA Dangerous Good Regulations (the IATA Regulations) are the most comprehensive and, in general, include the requirements of the other regulations.

- The IATA Regulations include operational requirements for airlines to accept and transport dangerous goods safely and efficiently.
- The IATA Regulations include a detailed list of individual articles and substances specifying the United Nations classification of each article or substance and their acceptability for air transport as well as the conditions for their transport.
- The IATA Regulations describe goods that:
    - have been identified as being too dangerous to be carried on any aircraft under any circumstances;
    - are forbidden under normal circumstances but may be carried with specific approvals from the States concerned;
    - are restricted to carriage on all cargo aircraft; and
    - can be safely carried on passenger aircraft provided certain requirements are met.
- In relation to goods that may be carried by air, the IATA Regulations prescribe minimum requirements that must be met in relation to:
    - packaging - to ensure the safe transport of dangerous goods by air.
    - packing Instructions - including a wide range of options for inner, outer and single packagings.
    - training - to ensure that all individuals involved in the preparation or transport of dangerous goods are properly trained to carry out their responsibilities. Depending on the job-function, this may entail only familiarisation training or may also include more detailed training in the intricacies of the IATA Regulations.
- the proper declaration of dangerous goods by the shipper – to ensure that all links in the transportation chain know what dangerous goods they are transporting, how to properly load and handle them and what to do if an incident or accident occurs either in-flight or on the ground.
- reporting of accidents or incidents - so that an investigation by the relevant authorities can establish the cause and take corrective action.

RELEVANT TRANSPORT AUTHORITIES

- **Post**

‘Postal Guide’ available from Australia Post

• **Air**

Civil Aviation Safety Authority
GPO Box 2005
CANBERRA ACT 2601
Telephone (national enquiries): 131 757
Relevant Codes are:

- ICAO Technical Instructions for Safe Transport of Dangerous Goods by Air; and
- The International Air Transport Association (IATA), *Dangerous Goods Regulations*.

These documents are available from Hunter Publications, 58A Gipps Street, Collingwood, Victoria 3066 (telephone: (03) 9417 5361).

• **Sea**

Ship and Personal Safety Services
Australian Maritime Safety Authority
GPO Box 2180
CANBERRA ACT 2601
Telephone: (02) 6279 5023
Facsimile: (02) 6279 5966
or State offices of the Department.
Relevant code: International Maritime Dangerous Goods Code.

• **Road and Rail**

Regulation Policy and Projects Section
Road User Branch
Department of Transport
GPO Box 594
CANBERRA ACT 2601
Telephone: (02) 6274 7111
Facsimile: (02) 6274 6721
or State Offices of the Department.
Relevant code: Australian Code for the Transport of Dangerous Goods by Road and Rail.

APPENDIX 3 GLOSSARY OF TERMS AND ACRONYMS

accredited organisation	an organisation accredited under Division 3 of Part 7 of the Act. For more information see the *Guidelines for the Accreditation of Organisation*s
the Act	the Commonwealth *Gene Technology Act 2000*
AS/NZS 2243.3:2002	Australian/New Zealand Standard 2243.3:2002 *Safety in laboratories – Part 3: Microbiological aspects and containment facilities, published in the year 2002.*
BSC	Biological Safety Cabinet
certification	certification by the Regulator of a facility to a particular containment level under the Act
closed footwear	footwear that completely covers the foot, including the heel
dealings or deal with	has the same meaning as in the Act "deal with", in relation to a GMO, means the following: (a) conduct experiments with the GMO; (b) make, develop, produce or manufacture the GMO; (c) breed the GMO; (d) propagate the GMO; (e) use the GMO in the course of manufacture of a thing that is not the GMO; (f) grow, raise or culture the GMO; (g) import the GMO; and includes the possession, supply, use, transport or disposal of the GMO for the purposes of, or in the course of, a dealing mentioned in any of the paragraphs (a) to (g)
environment	has the same meaning as in the Act "environment" includes: (a) ecosystems and their constituent parts; (b) natural and physical resources; and (c) the qualities and characteristics of locations, places and areas
EPA-approved	for the purposes of these Guidelines "EPA-approved" means approved by the relevant government authority in the jurisdiction in which an activity is occurring

facility	has the same meaning as in the Act “facility” includes, but is not limited to, the following: (a) a building or part of a building; (b) a laboratory; (c) an aviary; (d) a glasshouse; (e) an insectary; (f) an animal house; (g) an aquarium or tank
GM	genetically modified
GMO	genetically modified organism
housing (of plants or animals)	for the purposes of these Guidelines this means the sheltering, lodging or growing of animals or plants for the majority of their life during the work of the GMO dealing
IBC	Institutional Biosafety Committee
inspection report	a report on a facility's compliance with the containment requirements in these Guidelines
LSOC	Laboratory Safety Officers Committee
NATA	National Association of Testing Authorities
OGTR	Office of the Gene Technology Regulator
PC1	Physical Containment Level 1(the lowest containment level)
PC2	Physical Containment Level 2
PC3	Physical Containment Level 3
PC4	Physical Containment Level 4 (the highest containment level)
procedures	for the purposes of these Guidelines, the meaning of “procedures” shall include any activity involving work with organisms
the Record	the record of GMO and GM Product Dealings, as mentioned in section 138 of the Act
the Regulations	the Commonwealth *Gene Technology Regulations 2001*
the Regulator	the Gene Technology Regulator appointed under Section 118 of the Act

ATTACHMENT 1: PEST CONTROL PROGRAM

PC2 Laboratory Pest Control Program

Lab Location:

Date	Reported By	Pest Report Details	Beims	Action Taken

ATTACHMENT 2: STAFF TRAINING

All new staff that will be working in the PC2 laboratory will require training in PC2 requirements and in GMO handling. New staff induction protocol will require staff to read this Manual and to sign off in this attachment. This sign off will be repeated every 12 months to ensure staff are familiar with all updates to the Manual. In addition, new staff will be closely supervised in all laboratory procedures by a senior staff person for a minimum of two weeks to ensure competency in PC2 procedures. Finally, there shall be an annual review of laboratory procedures in the form of Seminars given by trained staff or by representatives of the OGTR.

By signing below the following staff members have indicated that they have read and have agreed to comply with the procedures outlined in this manual:

NAME:	**SIGNED:**	**DATE:**

By signing below the following staff members have indicated that they have read and have agreed to comply with the procedures outlined in this manual:

NAME:	**SIGNED:**	**DATE:**

Authorisation Stamp

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