

SAFETY IN LABORATORIES



WORKING MICROBIOLOGICALLY

The basic approach to working with microorganisms is to regard them as potential pathogens and to handle them with standard microbiological techniques.

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PREFACE

This document specifically sets out requirements, responsibilities and general guidelines which promote safety in school laboratories where microorganisms (protozoa, fungi, free-living bacteria, cell-dependent bacteria and viruses) and genetically modified organisms (GMOs) are handled. The document is based on:

- Australian/New Zealand Standard TM AS/NZS 2243.3:2002 (Safety in laboratories, Part 3: Microbiological aspects and containment facilities). Available from www.standards.org.au
- The Office of the Gene Technology Regulator (OGTR) - Handbook on the Regulation of Gene Technology in Australia. Available from www.ogtr.gov.au
- The Queensland Department of Education Manual (DOEM) - Health and Safety Modules.
- Education Queensland, Aspects of Science Management: A Reference Manual for Schools. (1997).

In addition to the many problems commonly encountered in chemical laboratories, laboratories in which microbiological procedures and techniques are practiced can pose the following specific problems:

- Potential for infection of teaching staff, laboratory technical staff, and students.
- Cross-contamination of cultures.
- Contamination with adventitious microorganisms.

This document is intended to assist in addressing the obligations placed on schools, teachers and laboratory technical staff as employees, to provide duty of care for themselves, students and others in the workplace, under occupational health and safety legislation.

This document in itself is not an approved set of guidelines, but rather a synthesis of recommendations, guidelines and procedures that should form the basis for informed decisions as to whether an adequate level of safety can be provided to comply with duty of care. This document should be read in conjunction with any and all approved guidelines and legislation.

The author does not accept responsibility for the accuracy of the contents of this document, nor accept responsibility for the application of the contents. It is incumbent on individuals to conduct their own risk assessment for each individual circumstance, and to adopt standard operating procedures that minimize risks to all participants.

The term 'shall' is used to indicate requirements that need to be met for compliance with the contents of the document. The term 'should' indicates a recommendation.

ORGANIZATION, RESPONSIBILITY and HEALTH MANAGEMENT

SCHOOL MANAGEMENT RESPONSIBILITY

As an overall principle under occupational health and safety (OHS) laws in the States, Territories and Commonwealth of Australia, the employer is responsible for ensuring that the workplace is safe and free from risks to health. School management shall provide staff with a policy statement on laboratory safety that recognizes the special hazards associated with school laboratories where microbiological procedures and techniques are practiced.

No teaching or operational work with potential biohazards shall be undertaken in school laboratories until a risk assessment of the work is conducted and it is demonstrated that any hazards are controlled. In the case of genetically modified organisms, all 'dealings' must either be licensed, notified or exempt.

Schools should appoint a Safety Officer responsible for microbiology as a member of their Safety Committee. This individual should:

- Advise management on the need for safety equipment.
- Coordinate and organize the safety program.
- Prepare a specific section for the school's laboratory safety manual, detailing safe procedures relevant to the type of work conducted in the laboratory.
- Investigate accidents in the laboratory.
- Ensure that new members of staff are given clear directions on safety matters as part of their induction.
- Train and advise staff in safety regulations and decontamination procedures.

LABORATORY SUPERVISOR/ HEAD OF DEPARTMENT (HOD) RESPONSIBILITY

Overall, the HOD shall ensure that safe procedures are documented and put into practice. The HOD shall:

- Implement initial and continuing staff training programs.
- Advise all staff of the potential risk of occupational exposure to microorganisms.
- Ensure the provision of personal protective equipment for laboratory users.
- Report incidents involving genetically modified organisms or recombinant DNA to the OGTR.
- Ensure maintenance is carried out in accordance with safe procedures.
- Issue any special instructions to cleaning contractors necessary for the cleaning of teaching laboratories and preparation areas.
- Ensure that casual visitors do not have unrestricted access to the laboratory and preparation areas.
- Ensure that a readily accessible, appropriately maintained first aid kit is provided in an unlocked and clearly labeled container.

PERSONAL RESPONSIBILITY

All laboratory work shall be carried out with regard to the safety of laboratory occupants. The following requirements apply to all laboratory users:

- Individuals shall be familiar with the recommendations and requirements in the laboratory safety manual.
- Individuals shall be familiar with, and shall use, the appropriate safety equipment provided.
- Individuals shall ensure that minor cuts and abrasions, which provide routes for infection from contaminated surfaces, should be adequately covered and kept dry.
- Individuals shall report all injuries that occur in the workplace to the laboratory supervisor as soon as possible, and they shall be documented.
- Individuals who are immuno-suppressed, immuno-compromised, or otherwise unduly vulnerable to infection (such as persons who are pregnant or diabetic) should inform the supervisor of their condition so that appropriate action may be taken.

DEGREE OF HAZARD FROM MICROORGANISMS

GENERAL

All work with microorganisms requires the use of standard techniques to minimize risk to staff, students and the environment. Such techniques also maintain the purity of strains of isolates in the laboratory.

The basic approach to working with microorganisms is to regard them as potential pathogens.

Microorganisms are classified by risk group based on past experience with laboratory infections.

Laboratories, physical containment equipment, laboratory design and procedures to be followed are classified according to the risk group of the microorganisms being considered.

CLASSIFICATION OF INFECTIVE MICROORGANISMS BY RISK GROUP

Classifications of microorganisms according to degree of risk is based on the pathogenicity of the agent, the mode of transmission and host range of the agent, the availability of effective preventive measures, and the availability of effective treatment:

- (a) *Risk group 1 (low individual and community risk)*
- (b) *Risk group 2 (moderate individual risk, limited community risk)*
- (c) *Risk Group 3 (high individual risk, limited community risk)*
- (a) *Risk Group 4 (high individual and community risk)*

Only microorganisms from Risk Group 1 (unlikely to cause human, plant or animal disease) are suitable for use in school laboratories. The collection/isolation of microorganisms or material likely to contain microorganisms that may be associated with animal, plant or human disease of moderate severity (Risk Group 2 microorganisms) should not be undertaken.

No table is provided for microorganisms belonging to Risk Group 1, as the number of relevant microorganisms is too 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 microorganisms *Acetobacter* sp and brewer's and baker's yeast. Others include *Eschericia coli* (K12), *Staphylococcus albus* (*epidermidis*), *Sarcina lutea* (*Micrococcus luteus*), *Bacillus subtilis*, *Rhodospirillum rubrum* and *Pseudomonas fluorescens*. Some microorganisms that are regarded as part of the normal flora of humans or animals may be pathogenic for immuno-compromised/immuno-suppressed individuals.

NOTE:

- Many parasites are regarded as Risk Group 2, with respect to their infective stages.
- The majority of plant pathogens may be classified as Risk Group 1. Persons using plants in genetic manipulation work should consult the OGTR regulations and guidelines.
- Plant and fish pathogens are an infrequent cause of disease in humans but should be handled with good microbiological practices to avoid liberation into the environment

CLASSIFICATION OF LABORATORIES, PRACTICES and PROCEDURES

PHYSICAL CONTAINMENT LEVEL 1 (PC1)

This level of facility with its practices and equipment is appropriate for school laboratories. A Physical Containment Level 1 laboratory is suitable for work with microorganisms in Risk Group 1 where laboratory users can be adequately protected by standard laboratory practice. Work may be carried out on the open bench. Specimens that have been inactivated or fixed may be handled in a PC1 laboratory.

All work done in a PC1 laboratory shall follow the procedures prescribed for that level of physical containment.

> PC1 LABORATORY FACILITIES:

- Bench tops shall be made of an impervious and heat resistant material.
- Seats shall be of smooth impervious material to facilitate cleaning.
- Washbasins with potable hot and cold water services shall be provided inside each laboratory room, near the exit.
- Open spaces between and under benches, cabinets and equipment shall be accessible for cleaning.
- Fire control systems shall meet local, State, or Federal regulations.

> PERSONAL PROTECTIVE CLOTHING AND EQUIPMENT (PPCE):

- Protective clothing that affords protection to the front part of the body shall be worn within the laboratory. Long-sleeved laboratory coats, back opening gowns or coveralls should be used.
- Closed footwear incorporating impervious leather uppers shall be worn.
- Safety glasses face shields and other protective devices shall be worn where appropriate to protect eyes and face from splashes and other hazards. Contact lenses do not provide protection against laboratory hazards.

> WORK PRACTICES:

- Food or drink for personal consumption shall not be brought into the laboratory or stored in laboratory refrigerators. Eating, drinking, smoking, shaving and the application of cosmetics shall be prohibited in laboratories.
- Long hair shall be tied back.
- All emergency and safety equipment shall be kept and maintained in accordance with the manufacturer's instructions.
- All safety equipment shall remain readily accessible to laboratory users at all times.
- All work hazards shall be identified, assessed for their risk, and controls implemented where necessary.
- Skin areas that come into contact with chemicals shall be washed, irrespective of concentration.
- Mouth pipetting shall not be allowed.

- All cultures shall be clearly identified and dated. Cultures shall not be stored for long periods on the bench. They shall be transferred to a dedicated storage area, such as a refrigerator or part of a cold room.
- Disposable loops are preferable to flaming wire loops, as they do not generate aerosols.
- Care shall be taken to minimize the production of aerosols.
- Precautions shall be taken to ensure that reading and writing materials do not become contaminated.
- Labels shall not be moistened with the tongue. Use self-adhesive labels.
- All spills shall be cleaned up immediately and the area decontaminated.
- Workbenches shall be decontaminated at least daily and after each teaching session is completed.
- Specialized wastes shall be segregated at point of discard, decontaminated and disposed of according to local regulations.
- Protective clothing and equipment shall be removed and stored in facilities provided.
- Hands and fingernails shall be thoroughly washed before leaving the laboratory.

LABORATORIES FOR GENETIC MANIPULATION

The most common example of genetic manipulation in schools is bacterial transformation. All work with genetically modified organisms conducted in Australia is affected by the *Gene Technology Act 2000* and corresponding state laws. Under Australian law, all 'dealings' with GMOs must either be licensed, notified or exempt. Work undertaken by students in schools will most likely *not* require a licence and will probably be exempt.

To be exempt from licensing, dealings must:

- Meet the definition of an 'exempt dealing' (see abridged table below).
- Be conducted in a laboratory that meets the minimum conditions for Physical Containment Level 1 (see above).
- Not release viable GMOs into the outside environment.

If **all** of these conditions can't be met, approval from the Gene Technology Regulator must be sought.

TABLE 1: DEALINGS EXEMPT FROM LICENSING (ABRIDGED)

Class	Host	Vector
Bacteria	<i>Escherichia coli</i> K12 or <i>E. coli</i> B - any derivative that does not contain: (a) Conjugative or generalised transducing phages; or (b) Genes able to complement the conjugation defect in a non-conjugative plasmid	1. Non-conjugative plasmids 2. Bacteriophage (a) lambda (b) lambdoid (c) Fd or F1 (e.g. M13)

A record should be kept of the type of GMOs used and the nature of the experiment undertaken. In the event of an unintentional release of a GMO, the Office of the Gene Technology Regulator must be notified as soon as possible on 1800 181 030 and advice sought on managing the incident.

GENERAL LABORATORY PROCEDURES

WORK AREAS

Segregation of Special Areas

Separate areas shall be provided for the following:

- Preparation of media.
- Holding of materials awaiting sterilization.
- Sterilization.
- Storage of sterile articles.
- Administrative work.

CLEANING

Work areas shall be kept tidy, free of materials not being used and free from physical hazards that might cause spillages or breakages. Benches and work surfaces shall be cleared at the end of each teaching session and then disinfected. Items for sterilization shall be collected at the end of each teaching session.

Wet mopping, with a solution having detergent properties, is the most practical method of cleaning floors. Vacuum cleaning shall only be used where a vacuum cleaner has a HEPA filter fitted to the exhaust. A household type vacuum cleaner, which produces aerosols, shall not be used in a microbiology laboratory. Brooms shall not be used for sweeping, as they produce airborne dust that can increase contamination of work in the laboratory.

Walls shall be cleaned periodically, or when visibly dirty, by washing with a detergent solution. Open shelves collect dust and shall be cleaned routinely.

Apparatus such as centrifuges, water baths, incubators, refrigerators, deep freeze cabinets and liquid nitrogen storage vessels shall be cleaned and, where appropriate, disinfected at regular intervals and before being sent for repair or disposal.

WASTE DISPOSAL

Collection

Laboratory wastes shall be collected in segregated containers, clearly identified according to the following categories:

- *Non-infectious material* Waste paper, plastics, and paper products shall be collected in a single layer plastic bag.
- *Sharps* These can include syringes with needles, broken glass, and scalpel blades and shall be collected in a rigid, puncture-proof container.
- *Infectious material* Sample remains, used Petri dishes, culture bottles, disposable equipment and used gloves shall be collected in a robust plastic container displaying the biohazard symbol.

Treatment and Disposal of Wastes

All unwanted wastes containing live microorganisms shall be either:

- Sterilized by pressure steam sterilization; or
- Treated by a chemical disinfectant (minimum of 30 minutes immersion but preferably overnight).

After sterilization or chemical treatment, solid waste can be disposed of in landfill, provided that such disposal complies with local regulations.

The following points shall be observed:

- All waste involving genetically manipulated organisms shall be disposed of in accordance with the requirements of the OGTR.
- General uncontaminated laboratory wastes may be disposed of in the same manner as household waste.

PRESSURE STEAM STERILIZERS

Pressure steam sterilization (autoclaving) is the most reliable means of decontamination. Pressure steam sterilizers operate at high pressures and temperatures, and appropriate measures shall be taken for personnel safety.

Sufficient penetration time should be allowed for all parts of the load to reach the desired temperature. Minimum sterilization times after attainment of temperature shall be –

- (a) 15 minutes at 121°C; or
- (b) 4 minutes at 134°C.

Some visual indicators, such as sensitive papers or tapes, give only an indication that the sterilizer load has reached a specified temperature and do not give an indication of how long the load has been exposed to that temperature. Other chemical indicators progressively change colour with the time exposed at specified temperatures, and their use is recommended as they give an immediate indication of the efficacy of treatment.

CHEMICAL DISINFECTANTS

While pressure steam sterilization is the most reliable means of decontamination, this method is not applicable in all situations and may not be readily accessible to all school laboratories. Chemical disinfection is often the only practical method available to schools for decontamination for large spaces or surface areas and for heat-labile materials or equipment. See Table 2.

Microorganisms vary in their susceptibility to chemical disinfectants. Working solutions of disinfectants should be frequently replaced with freshly prepared dilutions from stock solutions. The containers or dispensers used should also be emptied between batches and their contents not merely 'topped up'.

TABLE 2: RECOMMENDED APPLICATIONS FOR CHEMICAL DISINFECTANTS

Site or Equipment	Method
Benches and surfaces (not obviously contaminated)	Swabbed with alcohol e.g. 70% w/w (80% v/v) ethyl or 60-70% v/v isopropyl -
Discard containers	Chlorine disinfectant at 2000 – 2500 p.p.m. (0.2 – 0.25%), freshly prepared and changed daily. 'Milton' is an excellent alternative.
Hand disinfection	Chlorhexidine (0.5 – 4% w/v) in alcoholic formulations for 2 minutes. Isopropyl (60 – 70% v/v) or ethyl alcohol (80% v/v) with emollients or Povidone-iodine (0.75 – 1% as I) for 2 minutes.
Hygienic handwash	Chlorhexidine (4% w/v) in detergent or alcoholic formulations/detergent cleansers or soap, all for 15 seconds

Spills of bacterial cultures	Synthetic phenolics/Iodophor (dilute according to manufacturer's instructions) for 10 minutes. High concentration chlorine disinfectant for 10 minutes
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LABORATORY SPILLS

A minimally hazardous material (Risk Group 1) that is spilled without generating significant aerosol may be cleaned up with a paper towel soaked with an effective chemical disinfectant.

- (a) Put on appropriate protective clothing such as gloves and gowns.
- (b) Place absorbent material wetted with suitable disinfectant over the spill. Alternatively, proprietary absorbent materials which release hypochlorite may be used. Allow approximately 10 minutes to effect disinfection. Remove any sharp objects with forceps and discard as contaminated sharps.
- (c) Use the same disinfectant solution to wipe over the area likely to have been contaminated.
- (d) Carefully mop up the spill and disinfection solution, and transfer all contaminated materials for disposal.
- (e) Remove protective clothing and wash hands.

LABCOATS/APRONS

AS/NZS 2243.1:1997 Safety in Laboratories Part 1 General 4.2 Use of Personal Protective Equipment 4.2.2 Clothing- "Note: Contaminated protective clothing should not be laundered domestically."

GLOVES

Contamination of hands may occur when laboratory procedures are performed. Disposable latex, nitrile or vinyl surgical-type gloves should be used where appropriate. Gloves shall be removed and hands thoroughly washed before leaving the laboratory. Used gloves shall be discarded with infected laboratory waste.

Powder-free gloves may help to avoid allergic reactions such as dermatitis.

BUNSEN BURNERS

The use of Bunsen burners should be minimized wherever possible. Disposable inoculating loops, spreaders and pipettes should be considered as safer alternatives.

TRANSPORT OF BIOLOGICAL MATERIALS

Procedures have been established for the safe transport of biological materials by air, rail and road. Different packaging and transport arrangements apply depending on the infectivity of the material, or whether they contain genetically modified microorganisms. It is the responsibility of the sender to ensure compliance with all packaging and transport regulations.

Biological materials known not to contain infectious agents may be transported without restrictions.

A biological product containing organisms in Risk Group 1 and those known not to contain microorganisms from Risk Groups 2, 3 or 4 may be transported without restriction.

All materials that are genetically modified but thought unlikely to be infectious for animals or humans shall be packaged according to Packing Instructions No. 913 of the IATA *Dangerous Goods Regulations*.