SMCE | Mechanical & Chemical Engineering

Biotechnology Safety Manual

JUNE 2013

Table of Contents

Acknowledgement

Thanks to Ph.D. student Xiaojuan Duan for permission to use her image on the front cover.

Preface

This Biotechnology Safety Manual is provided as a training guide to engineering researchers and students working in the biological area. It covers essential areas of safety compliance, but is not intended as a definitive source of information when working with biological organisms.

The content of this manual is aligned with UWA biological safety policies, procedures and guidelines as well as relevant information from AS/NZS 2243.3.2010. In addition, the manual provides a valuable information source to engineers who do not have a strong background in biological science and provides numerous references and links to other areas where more detailed information can be sourced.

This document, in conjunction with other training materials, assists Project Supervisors or Chief Investigators to meet their training obligations under current and future Work Health and Safety (WHS) legislation. AS/NZS 2243.3.2010 should always be consulted as a definitive guide when the requirement for information exceeds the scope of this document.

1. INTRODUCTION TO LABORATORY SAFETY

The University of WA has a legal and moral responsibility to its staff and students to provide a hazard free workplace. To meet the requirement of the Occupational Safety and Health Act and Regulations, as well as the new model Work Health and Safety (WHS) harmonised system - UWA has put in place its own Work Health and Safety Policy, procedures and guidelines. It is the responsibility of all Faculties, Schools and Centres to ensure these principles are complied with.

Everyone in the Faculty of Engineering, Computing and Mathematics (FECM), including students, visitors and contractors, has the responsibility to abide by the safety policies and procedures of the University to ensure a hazard free environment and safe workplace. The degree of responsibility is related to the level of authority and control that a particular individual has in the workplace. Under the WHS Act, Officers of a business or undertaking are obliged to exercise Due Diligence to ensure the organisation meets its work health and safety obligations, while Workers have an obligation to take Reasonable Care to protect themselves and others from injury.

1.1 Requirements for Working in Laboratories

Staff, students and visitors should be aware of the following requirements before being permitted to work in a laboratory:

- **Project Induction and Training** - No one is permitted to work unsupervised in laboratories or workshops unless they have received an Induction from their supervisor advising them of all specific hazards relating to the area and received information, instruction and training in the assigned task to ensure competency. Documented evidence is required that this process has been followed. Refer to the UWA Safety Management System toolkit located at:

  http://www.safety.uwa.edu.au/management

- **Safety Footwear** - no person will be permitted to enter any laboratory, with the exception of computer laboratories, unless they are wearing suitable enclosed footwear. Sandals, thongs and open shoes are not regarded as acceptable footwear;

- **Eye Protection** - this is compulsory in all designated areas such as workshops and chemical laboratories where the risk of serious eye injury is highest, but may apply to other areas. Signs displayed at entrances will identify areas requiring the use of eye protection;

- **Normal Working Hours** – normal working hours for UWA are 8:00 am to 5:00 pm with the exception of weekends and public holidays;
- **Undergraduate Students** – are not permitted to work alone between the hours of 5:00 p.m. and 8:00 a.m. or weekends and public holidays, unless supervised by a staff member or have a completed and approved Work Plan;
- **Staff, Visitors and Postgraduate Students** – are required to submit a Work Plan for approval by their supervisor or Head of School for all work outside of Core Working Hours. Work Plans define the boundaries of after-hours work that is permitted and ensure that potential hazards have been identified, assessed and controlled.

Certain operations may not be performed by staff, visitors or students outside of normal working hours according to Section 14.4 of the UWA Laboratory Code of Conduct:

http://www.safety.uwa.edu.au/topics/laboratory#Afterhours

Please read the contents of this Laboratory Safety Procedures manual thoroughly since it contains important information on the safety systems and procedures that apply to the area you will be working in. Ensure that the contents are completely understood before signing and returning the Biotechnology Laboratory Safety Record

Your cooperation will not only assist in preventing injuries, but also helps to maintain a safer and more efficient work environment for everyone.

2. LABORATORY SAFETY OVERVIEW

Safety is an important issue that needs to be brought to the attention of anyone commencing work in laboratories. The Laboratory Safety Manual contains important information on generic safety topics as well as specific hazards and safe work procedures for each laboratory group shown below.

FECM operates a diverse range of laboratories, which implies that staff and students may be exposed to numerous hazards. These include mechanical, electrical, radiation, chemical, gas explosion and toxicity as well as infection from biological agents. In order to maintain a safe working environment for everyone sharing the facility, it is essential that each person is familiar with the system of work and the safety procedures for individual areas.

2.1 Laboratory Categories

To assist in the preparation of specific safety procedures, SMCE laboratories have been divided into four groups:

- Chemical process laboratories;
- Gas process laboratories;
- Biotechnology laboratories;
- General purpose laboratories.

Anyone commencing work in a laboratory group will be required to receive a copy of the relevant Laboratory Safety Manual from their supervisor.

2.2 Laboratory Safety Procedures

Staff, visitors and students will receive a guided introduction to important areas of safety and laboratory procedures, and will be required to read and understand the contents of the specific Laboratory Safety Manual before signing the Laboratory Safety Record.

It is important to note that the laboratory safety procedures are only intended to supplement other forms of information, instruction and training provided by the individual's supervisor. Anyone working unsupervised in a laboratory is required to have demonstrated competence and documented evidence that they have received the required induction and training appropriate to the task and the hazards present in the work environment.

3. EMERGENCY PROCEDURES

3.1 Local Emergency Procedures

Each laboratory should have Emergency Procedures in place to deal adequately with the range of possible emergency scenarios that may occur or arise from the nature of the operations in that area. These procedures need to be documented and displayed prominently in the laboratory. All staff, visitors and students working in the area need to be made aware of these procedures as part of their induction.

The UWA Laboratory Emergency Response Procedures at the following link may be used as a reference to develop local emergency procedures for specific laboratories:

http://www.safety.uwa.edu.au/policies/laboratory_emergency_response

3.2 General Emergency Procedures

Follow UWA emergency procedures and contact UWA Security as follows:

- In case of emergency contact 2222 (internal)
- Alternatively;
- In case of emergency contact 6488 2222 (mobile)

3.3 Categories of General Emergency

A general emergency is defined as any of the following:

- Any emergency evacuation;
- Presence of smoke or fire;
- Personal injury;
- Personal threat;
- Bomb threat;
- Suspicious mail or package;
- Radioisotope or Biohazard spill;
- Chemical hazard/spill/gas leak.

For further information on each category please consult the UWA General Emergencies Procedures guide at the following link:

http://www.safety.uwa.edu.au/policies/emergency

> Always identify the requirements of people with special needs who may be working in your area.

3.4 Emergency Evacuation

Follow UWA emergency procedures and contact UWA Security as follows if the building alarm has not already been activated:

- In case of emergency contact 2222 (internal)

- Alternatively;

- In case of emergency contact 6488 2222 (mobile)

Always be aware of the nearest emergency exit and know your egress route to the emergency assembly point. Never re-enter the building unless authorised to do so.

Further information may be found at the following UWA link:

http://www.safety.uwa.edu.au/policies/emergency_fire_and_evacuation

4. BIOLOGICAL SAFETY COMPLIANCE

The safety requirements and procedures outlined in this document apply to all laboratories where work is carried out with human, animal, invertebrate, plant and soil samples or any other material that may contain infectious microorganisms. For work involving gene technology or genetic modification, the reader must refer to UWA Research Services for the relevant guidelines:

http://www.research.uwa.edu.au/staff/biological

It is the legal requirement to ensure that people working in biological areas are properly trained and fully comply with University policies, procedures and guidelines on biosafety and containment. It is important that anyone working with biological material is aware of potential hazards associated with pathogenic microorganisms or prions, and take all necessary precautions to exercise microbiological safety and prevent the spread of infection.

4.1 Biological Safety at UWA

UWA has prepared a Safety and Health Risk Register that maps the Legislation, Standards, Codes and Guidance into corresponding University Policies and Procedures. Please consult Sections 1.2(a), 1.2(b) and 1.2(c) from the link below and ensure you are familiar with each policy or procedure relevant to the facility or activity that is part of the planned activity:

http://www.safety.uwa.edu.au/management/risk-register

Areas engaging in biotechnology research need to be aware of the legislation that governs various types of work and the approvals or registrations required to authorise this work. Failure to comply with correct procedure can result in prosecution or forcible cessation of all similar work, including compliant work, at the University of Western Australia – your actions may adversely affect others who are conducting their work in a responsible way.

UWA Biological Safety operates under Research Services and has been set up to assist all biological research groups to meet their compliance obligations.

http://www.research.uwa.edu.au/staff/biological

- The Human Ethics Research Office is responsible to ensure the UWA researchers comply with relevant State and Commonwealth legislation, guidelines and codes relating to the use of human tissues, this includes organs, body fluids, tumours and biopsy samples.

  http://www.research.uwa.edu.au/staff/human-research/policies/human-tissue

- The Animal Ethics Office is responsible to ensure that UWA researchers comply with strict State and Commonwealth legislation, guidelines and codes involving research with live animals within Australia or overseas, and research using animal cadavers or tissues.

  http://www.research.uwa.edu.au/staff/animals

4.2 UWA Biosafety Approval

Heads of School, School Managers, Directors of Centres, Managers of Centres (and their Deputies) and Project leaders are reminded of the responsibility to remain aware of the activities of all staff, visitors and students working under their supervision and to consult with UWA Biological Safety – Research Services if they are carrying out work in any of the following areas:

- Genetically Modified Organisms (GMO);
- Non-GM Bio-hazardous Organisms;
- Security Sensitive Biological Agents;
- Importing, Exporting and Transporting;
- Collecting from the Wild.

Chief Investigators/Research Supervisors and researchers need to be aware that all work involving tissues of human or animal origin requires Ethics Approval – this cannot be issued retrospectively and non-compliance would be considered as research misconduct.

- **Human Ethics Approval** requires a formal submission to the Human Research Ethics Office signed by the Chief Investigator/Research Supervisor and the Head of School or Centre Director. Refer to the following link for further details of the process:

  http://www.research.uwa.edu.au/staff/human-research/approvals

  In addition, the applicant will need to complete a Risk Assessment and submit this to UWA Biological Safety - Research Service for approval. Refer to the following link for a copy of the form:

  http://www.research.uwa.edu.au/staff/animals/policies/?a=554498

- **Animal Ethics Approval** requires the applicant to complete the Animal Tissue/ Cadaver form (located under Miscellaneous) and submit to the Animal Ethics Office for approval:

  http://www.research.uwa.edu.au/staff/forms/animals

  In addition, the applicant will need to complete a Risk Assessment and submit this to UWA Biological Safety - Research Services for approval. Refer to the following link for a copy of the form:

  http://www.research.uwa.edu.au/staff/animals/policies/?a=554498

4.3 Compliance with Standards and Risk Assessment

UWA has the expectation that all biotechnology or biological work will be carried out in compliance with the guidelines of Australian/New Zealand Standard AS/NZS 2243.3:2010 that states for following:

> 'Safety in all laboratories is primarily a management responsibility, but is also an individual responsibility. It is the responsibility of management to provide and maintain protective equipment and containment areas, a policy relating to the safe work practices within a laboratory and to promote the training in, and institution of, those practices. It is the responsibility of the laboratory staff to carry out the safe work practices and to use protective equipment to minimise injury or prevent occupational illness, not only to themselves, but also to their colleagues. It is also the responsibility of managers to ensure that consideration is given to hazards to the general environment when dispensing or handling biological material. Staff training must be directed toward making safety an attitude of mind and an integral part of all laboratory procedures, so that a constant, purposeful control of the laboratory environment will result'.

Biological work may only commence following a documented Risk Assessment demonstrating that all hazards have been identified and controlled. This process must be reviewed regularly to ensure that any deviation in materials, method or process is reassessed and must be reviewed every five years. Refer to the UWA Biological Safety - Research Service link below for a copy of the Risk Assessment form:

http://www.research.uwa.edu.au/staff/animals/policies/?a=554498

Unlike other areas of risk assessment, a biological assessment needs to identify the specific pathogens present in the material or process, then determine the level of containment that is necessary before work can commence; for example, the engineering controls required may include the need for a Biological Safety Cabinet or a particular level of Physical Containment to be factored into the laboratory design and construction. Identification of pathogenic microorganisms requires knowledge of the principle groups of organisms that may be encountered, including laboratory analysis of materials.

Further information on Infectious Microorganisms may be found in Appendix A. Readers without an adequate understanding of these fundamental principles may fail to derive the full benefit of the information provided in this biological safety manual.

The Australian and New Zealand Standard AS/NZS 2243.3.2010 lists the following factors in the safety assessment for consideration before work commences:

- The microorganisms involved, their presence or absence in Australia, their source, risk group, volume, concentration, mode of transmission, host range, minimum infectious dose, vectors and the nature of the proposed work;
- The process and the equipment to be used;
- Storage requirements and safe handling between work areas and storage areas;
- The containment performance of the facility construction, including the seal quality of the facility, air pressure and directional control mechanisms, treatment and filtration of air leaving the facility and emergency backup systems, where applicable;
- The suitability of containment equipment such as biological safety cabinets, for the intended work;
- The training and experience of staff/workers with the particular organisms proposed for the work;
- Any health considerations for staff, i.e. vaccinations, baseline checks, medical monitoring;
- The capability to deal with a spill, such as by facility gaseous decontamination.

4.4 Roles and Responsibilities

The roles and responsibilities of Laboratory Supervisors and Laboratory Workers are defined as follows:

- The supervisor shall ensure that safe procedures are documented and put into practice. The supervisor shall implement initial and continuing training programs and ensure that workers receive adequate supervision, and that regular maintenance is carried out. The supervisor must also ensure that casual visitors do not have unrestricted access to the laboratory;
- Laboratory workers must familiarise themselves with the recommendations of the laboratory safety procedures manual and be familiar with, and use of the safety equipment provided;

- Laboratory workers should take full responsibility for decontamination prior to equipment maintenance or disposal and ensure correct procedures are followed for the disposal of biological materials.

4.5 Scope of this Document

The references, information and guidelines provided in this laboratory safety procedures manual relate to the safe handling and containment of microorganisms in all laboratories operating across the School of Mechanical and Chemical Engineering or at facilities external to the School where joint ventures or collaborations exist with other organisations or partners. This is further expanded to include work in the following areas:

- Research involving gene technology or genetic modification;
- Materials, tissues or samples of human, animal, plant or invertebrate origin including any work where specimens containing pathogenic microorganisms may be handled – this includes soil and water samples;
- Biochemical conversions that use the enzymes of bacteria and other microorganisms to break down biomass. In this case where microorganisms are used to perform the conversion process which may take the form of anaerobic digestion, fermentation or composting.

The definition of a microorganism is a microscopic organism that includes protozoa or other parasites, fungi, archaea, bacteria, unicellular algae, viruses and viroids.

5. BIOLOGICAL RISKS AND CONTAINMENT

In order to protect workers, the community and the environment from the spread of disease resulting from the unintentional transmission of microorganisms, standard microbiological techniques must be followed. It is standard practice when working with microorganisms to regard them as potentially pathogenic and follow standard procedures at all times. These principles in conjunction with good personal hygiene will minimise the risk of infection and prevent cross contamination between sources.

The underlying principles and requirements outlined below focus on the containment of microorganisms. Containment translates into the physical and operational structure of a biological facility and comprises the buildings, the services, the equipment and the work practices and procedures that are followed.

Pathogenic microorganisms vary greatly in their ability to infect humans, animals, plants and invertebrates, and to spread in the environment. This relates to factors such as dose, method of transmission, the physiology of the microorganism and the immune response from the host. As such, there is an international classification of microorganisms by Risk Group - this is then further adjusted on a country by country basis according to the environmental and socio-economic factors that prevail.

5.1 Classification of Microorganisms by Risk Group and Type

Infectious microorganism have been classified globally and allocated into one of four Risk Groups according to the degree of risk i.e. Risk Group 1 (least infectious) to Risk Group 4 (most infectious). World Health Organisation guidelines require that countries amend this list according to the pathogenicity of the microorganism, the mode of transmission and host range i.e. vectors of transmission, availability of effective preventative measures and the availability of effective treatment. The following classification applies to Australia and New Zealand for human and animal infectious microorganisms:

- **Risk Group 1** (low individual and community risk) – a microorganism that is unlikely to cause human or animal disease;

- **Risk Group 2** (moderate individual risk, low community risk – a microorganism that is unlikely to be a significant risk to laboratory workers, the community, livestock, or the environment. Laboratory exposure may cause infection, but effective treatment and preventative measures are available and the risk of spread is limited;

- **Risk Group 3** (high individual risk, limited to moderate community risk) – a microorganism that usually causes serious human or animal disease and may present a significant risk to laboratory workers. It could present a limited to moderate risk if spread in the community or the environment, but there are usually effective preventative measures or treatment available;

- **Risk Group 4** (high individual and community risk) – a microorganism that usually produces life-threatening human and animal diseases, represents a significant risk to laboratory workers and may be readily transmissible from one individual to another. Effective treatment and preventative measures are not usually available.

It should be noted that similar Risk Groups apply to Plant Infectious Microorganisms and Infectious Microorganisms carried by Invertebrates; these may be referenced from AS/NZ 2243.3:210. In the case of plants, infectious microorganisms cannot be spread to human and animal populations, but there is a high risk to the environment and other plants. Conversely, transmission of infections from invertebrates poses a significant risk to human and animal populations.

Risk grouping of microorganisms is also carried out according to type i.e. bacteria, viruses etc. Reference to the associated tables in AS/NZ 2243.3.2010 will allow biological workers to determine the risk rating of the microorganisms they are handling and determine the

appropriate level of containment. Reference to Appendix A of this safety manual will provide further generalised information on microorganism type.

5.2 Physical Containment Classifications

Containment of microbiological materials is essential in preventing personal exposure and transmission into the environment. Containment is based on the principle of the 'box-within-a-box' in which case the number of layers provided depend on the level of infection and the consequences of transmission. The three general descriptors are as follows:

- **Primary Containment Measures** form the constraints immediately surrounding the infectious material i.e. a biological safety cabinet or a containment vessel for storage or transportation;

- **Secondary Containment Measures** form the second layer of containment should infectious material be inadvertently released from the primary containment. In this case it may be the laboratory, including the electrical and mechanical services of that laboratory as well as the safe work practices. Alternatively, it may be a secondary transportation container;

- **Tertiary Containment Measures** form the outer layer of the containment and prevent transmission to the environment. This may take the form of isolation of buildings, restrictions on access, decontamination or removal of laboratory outer garments and disposal of potentially infected waste materials. In the case of storage or transportation, it may translate into outer packaging or additional containment layer.

Collectively, the level of physical containment provided must correspond at least to the risk group of the microorganism(s) identified in the risk assessment i.e. Physical Containment Level 1 (PC1) to Physical Containment Level 4 (PC4). For example, if the operations involve microorganisms from Risk Group 2, then the PC facility must be rated at PC2 or above.

AS/NZS 2243.3:2010 defines the four physical containment levels as follows:

- **Physical Containment Level 1 (PC1)** a laboratory or facility suitable for work with microorganisms where the hazard levels are low and where the laboratory or facility personnel can be adequately protected by standard laboratory practices. This level of laboratory or facility with its practices and equipment is usually suitable for student and undergraduate teaching laboratories. The organisms used should generally be classified as Risk Group 1. Specimens that have been inactivated or fixed may be handled in PC1 facilities;

- **Physical Containment Level 2 (PC2)** a laboratory or facility where its practices and equipment is applicable to research, diagnostic and other premises where work is carried out with microorganisms or material likely to contain microorganisms that are classified as Risk Group 2 microorganisms. If working with specimens containing microorganisms transmissible by the respiratory route or if the work produces a significant risk to humans or the environment from the production of infectious aerosols, a biological safety cabinet shall be used;

- **Physical Containment Level 3 (PC3)** a laboratory or facility where its practices and equipment is applicable to research, diagnostic and other premises where work is carried out with microorganisms or material likely to contain microorganisms that are classified as Risk Group 3 microorganisms. A PC3 laboratory or facility provides additional building features and services to minimise the risk of infection to individuals, the community and the environment;

- **Physical Containment Level 4 (PC4)** a laboratory or facility where its practices and equipment is applicable to work with microorganisms classified as Risk Group 4 microorganisms. A PC4 laboratory or facility is situated in a building separate from other laboratories or facilities or constructed as an isolated area within a building. The facility is maintained under negative pressure and includes secondary barriers such as sealable openings, airlocks or liquid disinfection barriers, a clothing-change and shower room contiguous to the laboratory or facility ventilation system, and air and liquid waste decontamination systems to prevent the escape of microorganisms to the environment.

  A PC4 laboratory or facility may be of two types; one where work is conducted in a Class III biological safety cabinet exhausting outside the facility or one where the work is conducted without being isolated and staff wear fully encapsulated positive pressure suits.

6. IMPLEMENTING CONTAINMENT

With knowledge of the classification of microorganisms by risk group and type, plus physical containment classifications, it is now possible to proceed to establish a compliant laboratory or work area. In the case of pre-existing facilities, the level of containment will need to be assessed against the Risk Group of the microorganism.

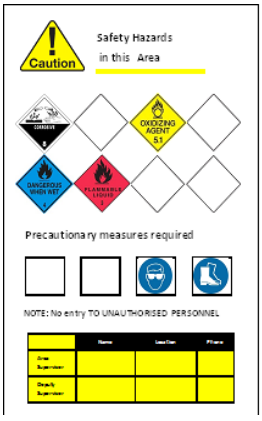
The following checklist must be followed:

- Risk assess the proposed work and determine the risk groups and types of microorganism that may be potentially present in the process or work;
- The physical containment level used should be at least the same level as the risk group or higher i.e. a Risk Group 1 microorganism requires at least a PC1 rated facility or higher;
- Consult Section 5 of AS/NZ 2243.3.2010 to determine the required compliance of Construction, Ventilation, Containment Equipment and Work Practices required;
- Consult Sections 6, 7 and 8 of AS/NZ 2243.3.2010 for containment requirements for animals, plants and invertebrates respectively.

Please note that a PC rating is not just a based on physical construction and provision of services, but also the work practices of the area. Inadequate work practices can result in PC2, PC3 or PC4 facilities being shut down under forcible cessation.

7. BIOLOGICAL HAZARD SIGNS

The entrance door(s) to microbiological laboratories of all physical containment levels must display a Microbiological Safety Sign in the format shown. It must show the standard biological hazard symbol as well as the physical containment classification and the laboratory type.



In addition, microbiological laboratories must display a UWA safety sign titled 'Safety Hazards In This Area' showing the name and contact details of the area supervisor and the deputy supervisor. This sign has provision for the display of safety symbols for Personal Protective Equipment (PPE) requirements in addition to Dangerous Goods Class symbols indicating the presence of hazardous chemicals and gas within the area.

All biological materials, samples and cultures require labelling and must display identification or description, the owner's name, the date of preparation and have a Dangerous Goods Class 6 Infectious Substance label attached.

Refrigerators and freezers that are used for the storage of biological specimens must also display a Biological Hazard warning sign.

8. WORK PRACTICES

Work practices are an essential element of containment. The required level of Physical Containment is achieved by construction, ventilation, containment equipment AND work practices.

First establish the required Physical Containment level for the process or work to be undertaken; then consult Section 5 of AS/NZS 2243.3.2010 to determine the work practices required for the containment level. It is unlikely in biotechnology that the Physical Containment level would exceed PC2.

There is considerable variation in the work practices required across the containment range PC1 to PC4; however, some common elements of good work practice are listed below:

- Compliance with AS2243.1 (Safety in Laboratories – Planning and Operational Aspects);
- Access restricted to authorised personnel only;
- All biological specimens must be regarded as potentially hazardous i.e. this includes animal specimens;
- No eating, drinking or storage thereof in the area, annexes or offices within the facility boundary;
- PPE to be worn at all times subject to local rules;
- Minimise exposure to biological materials, in particular aerosols from centrifuging or sonification and fungal spores from cultures – use ventilation extraction or biosafety cabinets whenever possible;
- Mouth pipetting is not permitted and delivery of liquid samples via forced expulsion should be avoided to prevent aerosol production;
- Identify and date all cultures and limit the time they remain on the bench before being transferred to permanent storage;
- Restrict the number of personal items taken into the work area and ensure that door handles, fridges, telephones, keyboards, reading and writing materials are regularly decontaminated;
- Work benches must be decontaminated daily and following work involving microorganisms;

- Domestic vacuum cleaners, brushes and brooms are not permitted to be used in biological areas. Only use vacuum cleaners fitted with a disposable bag and a HEPA filter on the exhaust;
- A procedure to clean up microbiological spills must be in place with appropriate training of staff to deal with incidents;
- A system for the segregation of biological waste (biological, radioactive, sharps) must be in place along with procedures for disposal in accordance with applicable regulations;
- Containment of biological hazards through the removal of laboratory gowns, PPE and hand decontamination before leaving biological areas is mandatory, including procedures for the transportation of biological samples;
- Any equipment transported from biological areas must be decontaminated prior to removal and maintenance or workshop staff must be advised of the potential hazard of disassembly;
- Biological areas must be decontaminated before maintenance staff are permitted to carry out repairs or maintenance and warnings must be issued about the potential hazards;
- Chemical handling and storage must be in accordance with AS/NZS 2243.10 (Safety in Laboratories – Storage of Chemicals).

9. INFECTION CONTROL

Whilst the likelihood of occupational exposure to serious pathogens for university researchers is not as high as for health care workers, awareness of infection control and regulatory procedures are generally lower resulting in a net tangible risk.

The effectiveness of an infection control program is dependent on the training of laboratory personnel and their fundamental understanding of the transmission modes of potentially infectious agents. This includes understanding the mechanisms associated with the propagation of bacterial, viral and fungal microorganisms that are responsible for most infections.

It is an established general safe practice to assume that all blood, blood products, body fluids and associated materials are potentially infectious and to treat them accordingly. By employing a consistent infection control program in conjunction with good personal hygiene, the spread of infection can be minimised or eliminated.

9.1 Acquired Laboratory Infections

Lungs are the most commonly infected organ and statistical data indicates that 80% of laboratory acquired infections are the result of exposure to aerosols containing infectious microorganisms that are produced by many common laboratory operations. Only 20% occur as the result of skin penetration injuries such as cuts or needle stick injuries.

Operations involving dissection, sectioning or manipulation of biological tissue will generate aerosols that can result in inhalation. The risk to researchers can be minimised by ensuring that surgical masks, or appropriate respirators fitted with P2 or P3 cartridges, are always worn during high risk procedures and that operations generating aerosol or dust (drilling) are carried out in a biological safety cabinet.

The risk of skin penetration is most common when disposing of or handling needles, syringes, scalpel blades and other sharp instruments. Skin puncture injuries are the primary cause of blood-borne disease in the health industry. This infection risk can be controlled by adhering to proper handling and disposal methods. Researchers should be aware of the suitability of tools and equipment for a particular purpose. Using industrial counterparts to save cost often exposes laboratory personnel to greater risk as the result of ineffective decontamination (serrated jaws of bench vices) or sharp corners and edges that may cause cuts and abrasions.

Another important factor in preventing the transmission of pathogens is personal hygiene; the single most effective measure is good hand hygiene. Compliance with basic hand hygiene practices is often suboptimal – this is evident by observing the practices of others. Behaviour is influenced by many factors including the ergonomics of equipment and process design, time constraints, awareness and poor personal hygiene.

Hands play a crucial role in the transmission of microorganisms during laboratory work since they are the common contact point between biological specimens, tools, equipment, exposure routes into the body, cross-contamination to other people (via door handles, light switches etc.) and to the environment outside of the laboratory.

The environment also has a significant role in the spread of pathogenic microorganisms since many can survive on the surface of objects and equipment for months unless decontamination is carried out regularly.

9.2 Infection Transmission Routes

Transmission identifies the exposure routes that are likely to result in laboratory acquired infections. Typically, four transmission paths are common:

- **Contact Transmission** is the principle and most frequent route of transmission and is divided into three sub-groups i.e. direct contact, indirect contact and droplet (aerosol) transmission. Direct contact transmission involves the transfer of the pathogen from a biological specimen to the researcher or to other personnel. Indirect contact transmission involves the transfer of the pathogen via an object such as an instrument, piece of equipment or even contaminated gloves. Droplet

(aerosol) transmission involves small droplets containing pathogens travelling in the air, over distances usually less than one metre, and being deposited on a person's body or inhaled into the lungs.

- **Airborne Transmission** occurs by the dissemination of either airborne droplet nuclei (<5 μm) or evaporated droplets containing pathogens that can remain suspended in the air for long periods of time, or dust particles released by drilling tissues. Pathogens are inhaled into the lungs and multiply rapidly in the alveoli allowing the infection to travel to any location in the body.

- **Vehicle Transmission** applies to pathogens that a propagated through routes such as blood, tissues and organs, or food and drink that if inappropriately brought into biological areas may become a carrier for disease.

- **Vector Transmission** is vector-borne when vectors such as flies, mosquitos or other vermin get into biological facilities and come into contact with biological specimens and propagate pathogens within the local environment or carry them outside.

9.3 Sterilisation, Decontamination and Cleaning

Identifying the types of microorganism that are likely to be present in a biological process are key elements in combating the release and exposure to infectious agents. In addition, engineering controls and safe work practices will further enhance the effectiveness of infection control. This will include the contribution of enhanced ventilation, ultraviolet irradiation and high efficiency particulate filters (HEPA) to remove pathogens from the environment or dilute their concentrations in air.

Since pathogenic microorganisms are not visible to the human eye it is not possible to determine how effective these measures are, therefore, additional precautions are required - these fall under the category of sterilisation, disinfection and cleaning.

- **Sterilisation** is a process for decontaminating media and equipment as well as decontaminating cultures and biological waste materials. In most cases it will employ high temperature, high pressure autoclave equipment to deactivate pathogens.

Readers should be aware that prions are resistant to denaturation because of their structural stability, and sterilising is not effective in deactivating prions. Unless the tertiary structure is completely destroyed they can be renatured back to the infective state. These properties make disposal very difficult.

Listed below are effective guidelines that should be followed for sterilisation of all instruments and devices used:

- Disassemble all mechanical devices and instruments and wipe individual parts with alcohol based wipes to removed visible deposits. Collect all solid debris and discarded wipes together and place in a biohazard bag for disposal;
- Spray each part or individual instruments with 1% solution sodium hypochlorite and leave for 10 minutes – leaving longer than 10 minutes may result in corrosion;
- Autoclave all items according to the autoclaving instructions;
- After sterilisation, wash in warm water and detergent (dishwashing liquid) and allow items to dry naturally;
- If corrosion is an issue, spray all metallic items with Inox;
- Store in a well-ventilated area with exposure to ultraviolet light as these are excellent natural decontamination sources.

A documented procedure should accompany this task and laboratory personnel need to be trained and assessed for competency.

- **Decontamination** refers to the disinfection of all benches, work surfaces and equipment that has or may have come into contact with potentially infectious material. All equipment and work surfaces should be wiped down with disinfectant at least once a day when work is in progress.

  The environment plays a big part in the spread of infection since pathogenic organisms may survive on shared objects for hours, days or weeks - regular disinfection is the principle method to prevent transmission.

  Remember that hands and protective gloves can easily become colonised with pathogenic organisms and often become the major source of transmission. The most common antimicrobials used for hand cleaning are alcohol and iodophor based solutions; these include products such as alcohols, chlorhexidine, chloroxylenol, hexachlorophene, iodine and iodophors. According to the World Health Organisation, alcohol based products are the most effective as they have the broadest antimicrobial spectrum and shortest application time of 20 to 30 seconds.

  The most effective method of decontamination in the work area is to be aware of typical pathogenic organisms that may be present from the materials and cultures used in the area or processes. In this case the most effective antimicrobial disinfecting agents may be selected for use. Alternatively, general

recommendations may be followed using broad spectrum antimicrobial cleaning agents.

These chemicals are recommended since they are known to kill HIV and hepatitis B if they are mixed and applied according to the manufacturer's instructions. Before using these chemicals it is important to have a copy of the relevant Material Safety Data Sheet (MSDS) and follow the safety instructions.

Sodium Hypochlorite

CAUTION: Sodium hypochlorite solution above 5% concentration is a Designated Hazardous Substance. It is corrosive and will cause severe burns to skin or lung damage if inhaled – care must be taken when handling and diluting concentrations of the substance.

Sodium hypochlorite is a safe and effective agent against HIV and Hepatitis A, B, C and D when left in contact with surfaces for at least 10 minutes. Use 10,000 ppm (1%) solution for areas soiled with blood or body fluids. Carry out a second wipe using 10,000-ppm (1%) solution and dispose of all wiping materials into the contaminated waste collection. For walls, benches, floor and objects that may be contaminated, but not visibly soiled, use a 0.5% solution. Finally, clean with water and a commercial detergent.

The effective strength of any chlorine solution decreases with time, therefore, fresh solutions should be prepared each day.

Sodium hypochlorite may be corrosive to some metal surfaces and should not be left in contact for more than 10 minutes. Wash metal objects in warm water and detergent and allow to dry. Spray with Inox if corrosion is likely to be a problem.

Ethanol

CAUTION: Ethanol is a Designated Hazardous Substance. It is highly flammable and should not be used near ignition sources. Low to moderate toxicity - avoid inhalation of vapour as well as skin or eye contact.

Ethanol used in a 70% solution with a contact time of 20 minutes is effective against a wide range of pathogens including HIV and Hepatitis A and B, but is ineffective against Hepatitis C and D. Industrial methylated spirits is an acceptable alternative since it has a 95% ethanol concentration.

Note that some disinfectants are more effective than others in deactivating specific pathogens. Also, be aware that some disinfectants are better suited for certain decontamination procedures. Written laboratory procedures should be developed for routine decontamination tasks, based on the effectiveness of particular antimicrobial disinfecting and the application method.

- **Cleaning** in this context refers to the general cleanliness and upkeep of the area or laboratory. Because of the biological classification of the area, especially laboratories designated PC2 or higher, cleaning contractors may be reluctant to enter to clean or even empty rubbish bins. Biological staff must establish protocols for general cleaning tasks, either directly with the cleaning service or make internal arrangements.

  Rubbish bins must be emptied regularly, floors must be kept clean and free from the accumulation of stored items. Housekeeping should be maintained on a regular basis and sharps disposal bins never allowed to overfill. Refrigerators and freezers must be defrosted regularly and unwanted samples or cultures disposed of immediately to prevent decomposition.

  For general cleaning operations it is recommended to use 5,000 ppm (0.5%) sodium hypochlorite solution.

9.4 Personal Protective Equipment

The specific requirements for Personal Protective Equipment (PPE) will be determined by a Risk Management approach. Entry to a biological area will usually have a minimum requirement, while specific procedures or operations may require additional PPE. The Risk Assessment will identify the hazards and the controls required and this information will be documented in the Method Statement or Standard Operating Procedures.

As a general rule, the requirement for additional PPE will increase commensurate with increasing Physical Containment level. Typical items of PPE listed below are regarded as the minimum requirement for biological laboratories and provide a barrier to minimise the risk of exposure to aerosols, splashes and accidental inoculation:

- Protective clothing that offers protection to the front of the body with preference to wear wrap-around theatre or laboratory gowns or coveralls;

- Safety glasses or face shields should be worn to protect the eyes and face from splashes and other hazards - contact lenses or glasses do not offer suitable protection;

- Surgical caps to cover the hair;

- Disposable latex or vinyl surgical gloves should be worn when working with biological materials to prevent contamination of the hands, but be aware that disposable gloves can also be colonised by disease pathogens and become a source of transmission;

- Stainless steel mesh gloves should be worn if there is a risk of cuts from sharp instruments during cutting or sawing operations;

- Cuts and scratches must be properly covered with waterproof dressings;

- Closed footwear should be worn at all time in laboratories and disposable overshoe covers worn during any sectioning procedures or when required by local rules;

- Respiratory protection is required for any operations likely to produce aerosols or dust, or involving the clean-up of spills - always wear a wrap-around surgical mask, or appropriate respirator during these operations and use a biological safety cabinet whenever possible.

Consult local working rules for all frequently executed tasks as these will list the PPE requirements specified in previous Risk Assessments, Standard Operating Procedures or Method Statements.

9.5 Microbiological Spills

Dealing effectively with microbiological spills is part of Infection Control and an extension of the principles of Sterilisation, Decontamination and Cleaning. Spill clean-up strategies will be determined by a Risk Management approach and will differ according to the Risk Group of the microorganisms and the Physical Containment level. Depending on these factors, in addition to the quantity of material being used, research groups must provide a strategy and equipment to carry out a clean-up. This may be done using expertise from within the project team or seeking the assistance of an external dedicated spill clean-up team.

Refer to Section 9 of AS/NZS 2243.3:2010 for details of the responses required to clean-up spills occurring inside Biological Safety Cabinets, centrifuges and within each of the Physical Containment levels. The information listed below is of a general nature to advise and assist researchers in planning the development of a spill response plan:

- Attend to spills onto the body or clothing as a first priority and remember that chemical showers are not suitable for biological decontamination as they may not have drains;

- Display a 'Biohazard' sign with 'DO NOT ENTER' when a spill occurs;

- Ensure that adequate quantities of freshly mixed disinfecting agents are available;

- Typical spills involve amounts of material from 1 ml or less to more than 100 ml. Ensure there is enough absorbent material and disposal containers to deal with the magnitude of the spill;

- Follow AS/NZS 2243.3.2010 instructions for clean-up depending if the spill is located inside or outside a biological safety cabinet or if it occurs inside other equipment such as a centrifuge;

- Typical spills will aggregate into a pool in addition to splashes and rivulets that may extend over some distance, as well as invisible airborne particles that form aerosols;

- Allow 30 minutes for aerosols to settle or use respiratory protection to prevent inhalation of potentially infectious droplets;
- Wear goggles to protect the mucus membranes of the eyes from aerosol infection and additional PPE according to the identified hazards of the task;
- Systematically collect and disinfect all surface areas, equipment or samples contaminated by spills and ensure disinfecting agents remain in contact with potentially infected items for at least 10 minutes;
- Disinfect and change gloves at regular intervals to prevent cross contamination as well as clothing that may become soiled during the clean-up process;
- If the spill has occurred inside a Biological Safety Cabinet then leave the cabinet running to expel aerosols and follow AS/NZS 2243.3.2010 instructions for spills occurring inside Biological Safety Cabinets;
- Have in place a documented list of clean-up materials and equipment suitable for the requirements of the area;
- Provide regular refresher training to the project team or consult with the external clean-up team on a regular basis according to the established response.

Complete and submit a UWA Confidential Incident/Injury Report form to the UWA Safety and Health Office within 24 hours.

9.6 Microbiological Exposure First Aid

Spills or splashes, depending on the risk of infection, will require an immediate response. Similarly, cuts, abrasions or needle stick injuries that may result in the blood-borne transmission of infectious microorganisms. The following steps must be followed:

- Immediately wash all cuts, abrasions and other puncture wounds with soap and water;
- Encourage bleeding to assist in the cleaning of the wound;
- Immediately irrigate eye splashes with the Emergency Eye Wash for 20 minutes;
- Immediately flush the mouth, nose, ears or face with water to remove splashes;
- Report the incident to your supervisor;
- Follow-up with a medical practitioner must be initiated immediately.

Complete and submit a UWA Confidential Incident/Injury Report form to the UWA Safety and Health Office within 24 hours.

9.7 Collection, Decontamination and Disposal of Microbiological Waste

All biological waste generated from microbiological facilities rated PC2 or higher shall be regarded as Clinical Waste and be managed in accordance with State and Commonwealth regulations i.e. Health Department of WA Guidelines for the Storage, Transport and Disposal of Medical Waste, AS/NZS 2243.3:2010 Microbiological Safety and Containment, and Management of Clinical and Related Waste AS/NZS 3816.

Collectively, these regulations require that clinical waste shall be clearly labelled, stored in a hygienic and safe way, decontaminated by chemical disinfection or preferably by autoclaving; then transported in a safe and compliant way for disposal by incineration.

UWA protocol requires that all sharps containers used are of an approved design compliant with AS/NZS 4301 and AS/NZS 4361 that are rigid, puncture-proof, leak proof and of sealable construction.

Biohazard bags of sufficient strength to safely contain the waste may be used for the segregation, collection and storage of clinical and related waste with the exception of sharps. Bags must conform to the colour coding and marking system for the different categories of waste listed below in accordance with Table 1 of AS/NZS 3816:1998.

Container or biohazard bag sizes should be selected according to the quantity and frequency of biological waste generation. In the interest of hygiene, biological waste should not be stored for long periods at the point of generation and it will need to be moved to a secure refrigerated storage area. Bags should not be filled to more than two thirds their capacity to allow a secure seal and prevent leakage.

- **Collection** of all clinical waste must be segregated into separate containers according to the following categories:

  - **Sharps** typically include scalpel blades, syringes, broken glassware or any other sharp object that are capable of cutting or penetrating the skin. Sharp objects may be contaminated with infectious, toxic or radioactive materials that substantially increase the risk potential of blood-borne transmission. Never remove needles from syringes or attempt to recap needles;

  - **Solid materials for disposal** comprises all biological materials of human, animal, invertebrate or plant origin, including samples, cultures, Petri dishes, potting mix, gloves and other items of disposable PPE. Larger items may be placed in wet strength bags and autoclaved prior to disposal;

- **Solid materials for reuse** comprise instruments, some glass and plastic laboratory ware, gowns and reusable PPE. Larger items may be placed in wet strength bags for disinfection or autoclaving;

- **Liquids for disposal** comprise body fluids, culture media, buffers, fluorescence and liquid effluent;

- **Co-mingled material** comprises biological material that is contaminated with either radioactive waste or chemical waste - collection and handling of this material must address the composite hazards;

- **Imported biological material** comprises all biological materials of human, animal, invertebrate or plant origin, including samples, cultures or soil samples. Consult with AQIS prior to disposal and comply with any conditions documented in the permit to import;

- **Cytotoxic materials for disposal** comprise any material that may be contaminated with cytotoxic drugs used in experiments. General waste contaminated with cytotoxic substances, including sharps, must be disposed of in commercially available cytotoxic waste bins;

Ethics protocols require that tissue of human origin is collected and stored separately from all other tissues and clearly identified in storage. Disposal is in accordance with the supply agreement and usually requires the material to be returned to the location of origin.

- **Radioactive material for disposal** may comprise solid or liquid waste. Solid waste must be sealed in a red plastic bag and placed in a cardboard radioactive waste box. All radioactive waste must be segregated and disposed of in accordance with the UWA guidelines documented at the following link:

  http://www.safety.uwa.edu.au/topics/radiation/radioactive-materials/waste-disposal

- **Decontamination** is a process carried out to deactivate the infectious properties of biological material according to the identified collection categories. Decontamination will be achieved by chemical disinfection (Section 9.3) or by autoclaving (pressure steam sterilisation method) with the exception of radioactive waste.

  The decontamination method employed for individual categories of biological waste will be determined by a Risk Management approach based on the risks associated with the project, the material and the facilities available. This will reflect in the local working rules and the training and competency of researchers to undertake this task;

- **Disposal** of all categories of biological waste listed in the collection categories above are to be disposed of by incineration using a high-temperature, high efficiency EPA-approved (Australia) incineration facility with the exception of:

  - **Radioactive material** should be disposed of in accordance with the UWA guidelines documented at the following link:

    http://www.safety.uwa.edu.au/topics/radiation/radioactive-materials/waste-disposal

  - **Cytotoxic material** comprising drugs and solutions in most cases can be safely deactivated by chemical methods at their point of generation or use. Refer to specific guidelines provided by the World Health Organisation (WHO) in the link below:

    http://www.who.int/docstore/water_sanitation_health/wastemanag/ch21.htm

  - **Chemical waste** should be separated from biological waste at the collection stage, classified as hazardous or non-hazardous and further segregated into a Dangerous Goods Class. Disposal of chemical waste may be organised via a licensed disposal contractor; alternatively, UWA arranges bi-annual chemical waste collections:

    http://www.safety.uwa.edu.au/topics/chemical/waste-service

Clinical waste disposal requires the use of yellow coloured bags or containers that display the bio-hazard symbol.

Disposal by incineration will require biotechnology project groups to establish contracts with commercial service providers and manage the costs associated with this process. Contact Buildings Safety and Space for other collaborative arrangements that may be available within UWA.

9.8 Transportation of Biological Materials

Transportation of biological materials includes importing (overseas and interstate), exporting (overseas and interstate) and local transportation on campus or between hospitals and research centres.

Project groups or individuals need to be aware of the permits, approvals and protocols that must be complied with for the six categories of biological materials listed in Section 13.3 of AS/NZS 2243.3:2010 Microbiological Safety and Containment. Furthermore, the transportation regulations established for the safe transport of biological materials by air, rail and road.

- **Importing Biological Materials** must comply with strict quarantine restrictions to preserve Australia's unique biosafety record and to protect the environment and agricultural industries:

  - **Overseas** requires approval from both Quarantine Western Australia (QWA) and also from the Australian Quarantine and Inspection Service (AQIS);

  - **Interstate** requires approval from Quarantine Western Australia (QWA).

If further details are required then contact UWA Biological Safety or refer to the following link for details:

http://www.research.uwa.edu.au/staff/biological/im-ex-trans-port

- **Exporting Biological Materials** requires the exporter to have the written consent of the receiver that they will accept delivery of the material; in addition there must be evidence that the receiver has obtained the necessary Import Approval/Permit:

  - **Overseas r**equires approval from both Quarantine Western Australia (QWA) and also from the Australian Quarantine and Inspection Service (AQIS);

  - **Interstate** requires both Quarantine Western Australia (QWA) and Quarantine Domestic that manages the transfer of biological materials across State and Territory borders.

- **Transportation** of Dangerous Goods Class 6.2 Infectious Substances is regulated by both international and national regulations that apply to the import and export of biological materials:

  - **Overseas** transport is regulated by the International Air Transport Association (IATA) Dangerous Goods regulations, the Australia Post Dangerous and Prohibited Goods and Packaging Post Guide, and the Australian Code for the Transport of Dangerous Goods by Road and Rail;

  - **Interstate** transport is regulated by the International Air Transport Association (IATA) Dangerous Goods regulations, the Australia Post Dangerous and Prohibited Goods and Packaging Post Guide, and the Australian Code for the Transport of Dangerous Goods by Road and Rail;

- **Local** transport across campus or locally between hospitals and research facilities must be in a double containment system. Both primary and secondary containers must be leak proof with both containers displaying the bio-hazard symbol. In addition, the primary specimen container should be sealable and the outside container must not be breakable. Containers must display identification of the contents, the owners name and the address. The material must be transported directly to its destination without deviation, by a person experienced in the hazards of the material.

9.9 Immunisation

The nature of work carried out in microbiological facilities may increase the risk of exposure to infectious agents. Immunisation will confer an artificially acquired immunity to allow the body's natural defence system to resist infection from bacteria and viruses. The University recognises its responsibility to make available appropriate immunisation to employees potentially at risk of exposure to vaccine preventable diseases.

All employees or students of non-immune status who are working in microbiological areas where there is an identified risk of infection are required to be vaccinated against these diseases and in most cases this would include Tetanus and Hepatitis B.

The cost of vaccination will be covered by the Faculty, School or Centre and employees or students have the right to refuse immunisation. Employees who refuse immunisation are required to complete a Declined Vaccination Form that is held on record. Students have the option to also decline completing the record form. In the case of individuals who decline vaccination a risk assessment should be carried out to determine if their respective work or study arrangements need to be changed to manage the risk. For further details on immunisation and responsibilities please refer to the following UWA Immunisation Guidelines:

http://www.safety.uwa.edu.au/topics/biological/immunisation

10. SECURITY SENSITIVE BIOLOGICAL AGENTS (SSBA)

The deliberate release of harmful biological agents such as viruses, bacteria, fungi and toxins has the potential to cause significant damage to human health, the environment and the Australian economy. The aim of the SSBA Regulatory Scheme is to limit the opportunities for acts of bio-terrorism or bio-crime to occur using harmful biological agents and to provide a legislative framework for managing the security of SSBAs.

The National Health Security Act 2007 and Regulations 2008 describes a national scheme for the regulation of SSBAs in Australia, and builds on Australia's obligations under the Biological and Toxin Weapons Convention (1975) and UN Security Council Resolution 1540 (2004).

The Department of Health and Ageing (DoHA) is the administrative body under the Act. Any institution that intends to store, or perform research with SSBAs must be registered with DoHA and the agent must be approved.

Tier 1 Agents	Tier 2 Agents
Abrin (reportable quantity 5 mg)	*African swine fever virus*
Bacillus anthracis (Anthraxvirulent strains)	*Capripoxvirus (Sheep pox virus and Goat pox virus)*
Botulinum toxin (reportable quantity 0.5 mg)	*Classical swine fever virus*
Ebolavirus	*Clostridium botulinum (Botulism; toxin-producing strains)*
Foot-and-mouth disease virus	*Francisella tularensis (Tularaemia)*
Highly pathogenic influenza virus, infecting humans	*Lumpy skin disease virus*
Marburgvirus	*Peste-des-petits-ruminants virus*
Ricin (reportable quantity 5 mg)	*Salmonella Typhi (Typhoid)*
Rinderpest virus	*Vibrio cholerae (Cholera) (serotypes O1 and O139 only)*
SARS coronavirus	*Yellow fever virus (non-vaccine strains)*
Variola virus (Smallpox)	
Yersinia pestis (Plague)	

List of Security Sensitive Biological Agents

If you are planning to use SSBA's then consult with UWA Research Services – Biological Safety and refer to the following link:

http://www.research.uwa.edu.au/staff/biological/ssba

11. BIOLOGICAL LABORATORY EQUIPMENT

It is common to find the following equipment located in microbiological laboratories - their regular maintenance and operational performance are key factors to the safety of personnel and the containment of pathogens.

11.1 Centrifuges

Centrifuges are commonly found in biological laboratories and are used to place objects in rotation around a fixed axis applying the force perpendicular to the axis. A centrifuge uses the sedimentation principle to separate particles of different density. Because of the nature of the materials processed in biological centrifuges at high rotational speed, there are both biological and mechanical hazards present.

If the tubes or buckets are not securely sealed, then there is a risk of the release of potentially hazardous aerosols or material. Alternatively, if regular maintenance checks are not carried out to identify corrosion, cracks or damage to buckets and rotors, then catastrophic failure can occur with explosive force capable of damaging laboratory infrastructure and causing serious injury.

The following guidelines should be considered when using centrifuges:

- UWA requires annual servicing of centrifuges by the manufacturer or local representative. Ensure that the centrifuge displays a current service label before use since a rotor failure can be catastrophic;
- Regular maintenance checks to determine the condition of rotors and buckets along with scheduled replacement according to the manufacturer's use by dates;
- Loaded tubes and buckets must be balanced before the equipment is operated;
- On completion of centrifuging, check for evidence of leaks from tubes or buckets before removing the contents. Refer to Section 9.5 Microbiological Spills for remedial action if spills have occurred;
- Carry out regular decontamination of the centrifuge bowl, rotor and the tubes/buckets in order to exercise good infection control and prevent cross contamination of samples;
- Ensure that tubes and buckets are loaded and unloaded in a Biological Safety Cabinet if they contain infectious materials of Risk Group 2 or higher;

- If a Biological Safety Cabinet is not available then wait 30 minutes before opening tubes or buckets to allowing time for potentially infectious aerosols to settle.

Centrifuges must only be used in accordance with the manufacturer's instructions and maintenance recommendations. Check the manufacturer's recommendations before using disinfectants on equipment parts.

Beware of exposure to harmful aerosols that may be released by this equipment.

11.2 Biological Safety Cabinets

Biological Safety Cabinets are required for work with Risk Group 2, 3 and 4 infectious microorganisms. The type of Biological Safety Cabinet (BSC) to be used is chosen according to the nature of the work and the toxicity of chemicals used with tissues. The purpose of the BSC is to provide an enclosed, ventilated laboratory workplace for the safe handling of biological materials that may contain infectious microorganisms.

Exhaust air in the BSC passes through HEPA-filters to ensure the removal of all microorganisms, including bacteria and viruses. All BSC's, with the exception of Class I, have a secondary function of preserving the sterility of the cabinet contents. Fume cupboards and BSC's are quite different in their filtering arrangements and must never be used as a substitute for the other in chemical or biological work.

Further information on the installation, use, exhaust and certification of BSC's may be found by referring to AS 2252.1 Biological Safety Cabinets Class I, AS 2252.2 Biological Safety Cabinets Class II and AS/NZS 2647 Biological Safety Cabinets – Installation and Use.

Three classes of BSC are available depending on the degree of bio-containment required.

- **Class I BSC** provides protection to both the operator and the laboratory environment from infectious microorganisms, but does not preserve the sterility of materials within the cabinet as unfiltered external air is drawn into the enclosure. This class is either ducted or unducted i.e. connected to the building exhaust or recirculated back into the laboratory respectively. They may not be used with materials that contain volatile hazardous chemicals unless they are configured for ducted exhaust operation. Their use is mostly restricted to manipulations with biological tissues or cultures and housing smaller related equipment that does not create air turbulence that may compromise containment.

- **Class II BSC** are the most common type of cabinet found in biological settings and provide protection for both the operator and the laboratory environment. In addition, the HEPA-filtering of supply air into the cabinet preserves the sterilisation of the cabinet contents. There are four types of BSC within Class II i.e. Type A1, Type A2, Type B1 and Type B2 - the most common type being Type A2. In Class II BSC's isolation from potentially infectious aerosols is maintained by a curtain of sterile air acting as a barrier to prevent contaminated air exiting the

cabinet. Class II cabinets may not be used with materials that contain volatile hazardous chemicals unless they are configured for ducted exhaust.

- **Class III BSC** would only be installed if maximum containment is required for work with Risk Group 4 infectious microorganisms. A Class III BSC is similar in construction and appearance to a Glove Box; it is a fully contained and airtight enclosure with gloves and sleeves allowing the operator to access the contents behind an isolation barrier. Materials are added or removed from the cabinet via an air-lock system to maintain microbiological isolation.

- **Maintenance and Testing** of containment efficiency and safety must be conducted annually and records kept. This process should also be followed after the installation of new equipment and after any modification, repair, relocation or HEPA filter change.

- **Preserving Containment** of Class I and Class II BSC's may be adversely affected by placing large pieces of equipment, such as centrifuges, inside the cabinet. Testing the BSC and equipment combination is essential to ensure that laminar airflow is maintained.

For further information on the maintenance and use of BSC's and Laminar Flow Cytotoxic Drug Safety Cabinets refer to Section 10.7 of AS/ANZ 2243.3 Microbiological Safety and Containment.

BSC's must be decontaminated before servicing and testing is undertaken. For cabinets used with Risk Group 1 microorganisms, follow the instructions in Section 9.3. For cabinets used with Risk Groups 2, 3 and 4 decontaminate with formaldehyde gas or an equivalent decontaminant. See AS/NZS 2647 Biological Safety Cabinets – Installation and Use.

11.3 Pressure Steam Sterilizers (Autoclave)

An Autoclave is a high pressure, high temperature sterilizer that is used for sterilizing equipment and materials. Operating at 121 °C for 15 minuted at 103 kPa or 134 °C for 3 minutes at 203 kPa using pressurised steam or superheated water, this equipment is able to deactivate biological pathogens such as bacteria, viruses, fungi and spores (excluding prions).

Traditionally, autoclaves have been used to disinfect laboratory equipment, glassware and surgical instruments, but there is a growing application for the sterilisation and deactivation of experimental waste both for pre-disposal (prior to incineration) or as a final disposal method. Currently, incineration or thermal oxidisation is mandated for the disposal of clinical waste and infectious or toxic waste.

The removal of air from the autoclave is an essential part of the sterilisation process since hot air is a very ineffective medium for sterilisation. The air removal method will depend of

the type of autoclave used and typical methods of air removal are downward displacement of air by steam or the use of an evacuation pump.

The effectiveness of the sterilisation cycle in deactivating infectious pathogens will depend on factors such as air trapped in the load, temperature distributions across the load, pressure and sterilisation time. Autoclave quality assurance is an essential part of the process and must take account of both temperature and time. One of the following monitoring methods should be used:

- **Chemical Indicators** that progressively change colour with exposure time at specific temperatures. Do not use indicators that only change colour when a specific temperature is reached. Chemical indicators must be used with each load.

- **Biological Indicators** contain spores of a heat-resistant bacterium that will germinate if the autoclave does not reach the correct temperature for the required time. This method requires incubation over several days before sterilisation assurance can be determined. Biological indicators should be used monthly at several locations selected from the coolest, densest positions in the load.

- **Bacterial Enzyme Indicators** can be used in preference to biological indicators since enzyme inactivation as the result of spore viability can be determined within minutes or hours. Bacterial enzyme indicators should be used monthly at several locations selected from the coolest, densest positions in the load.

A logbook recording the sterilisation cycles, temperature and time must be maintained, including all quality assurance records.

Autoclaves are potentially hazardous pieces of equipment since they operate at high temperature and pressure. Inhalation of vapour is an additional risk and all displaced air, steam or liquid shall be regarded as potentially contaminated. Records must be kept showing operator training, competency and authorisation to use the equipment.

Autoclaves are designated pressure vessels and it is a legislative requirement that they are registered with WorkSafe WA and records are kept of all inspection, maintenance and repairs. UWA requires that all pressure vessels are also registered with UWA Central Plant who will ensure that annual certification is carried out.

11.4 Fume Cupboards

It is not uncommon to find fume cupboards located in microbiological laboratories. This facility is useful for many activities associated with preparation of materials and the mixing and application of chemical substances. However, there should be awareness that fume cupboards must NOT be used for handling potentially infectious materials.

11.5 HEPA Filters

A High-Efficiency Particulate Filter (HEPA) is a type of filter commonly found in biological equipment or other processes where it is necessary to remove particles, sometimes as small as 100 nanometres, from air. In order to qualify as a HEPA filter there are certain performance standards that must be met and the filter grade is classified according to filtration efficiency. A further characteristic of HEPA filters is that there is minimum resistance to airflow and minimum corresponding pressure drop across the filter.

For biological containment Type 1, Class A filters must be used. These may have separators or can be separatorless, but they must have a minimum performance of Grade 2. The following performance grades are specified:

- **Grade 1** applies to a filter that has a filtration efficiency of not less than 99.97% at the rated airflow for nominal particles of 300 nanometre diameter;

- **Grade 2** applies to a filter that has a filtration efficiency of not less than 99.99% at the rated airflow for nominal particles of 300 nanometre diameter and leak tested in accordance with AS 1807.6;

- **Grade 3** applies to a filter that has a filtration efficiency of not less than 99.999% at the rated airflow for nominal particles of 300 nanometre diameter and leak tested in accordance with AS 1807.6;

- **Grade 4** applies to a filter that has a filtration efficiency of not less than 99.999% at the rated airflow for nominal particles of 120 nanometre diameter.

HEPA filters play a critical role in preventing the spread of infectious microorganisms and are effective against airborne viruses, bacteria and fungal spores and provide excellent protection against airborne disease transmission.

HEPA filters also have a vital role in bio-containment and infection control - since they are the principle barriers to containment, they need to be maintained properly and checked at regular intervals to ensure that standards are met. Increased use of concentrated acids or formaldehyde in microbiological areas is linked to the premature failure of HEPA filters.

Typical causes of filter failure include medium failure, blockage or gasket failure, while the damage to filter performance is most likely to occur in handling, installation and decontamination. Operation in a humid or wet environment may affect the retention characteristics of the filter, especially with aging. As filters age, their airflow resistance will increase and in biosafety applications it is not uncommon for a doubling of clean filter resistance to occur over the life of the filter.

The following considerations need to be addressed when choosing, testing and replacing HEPA filters in biological installations or on biological equipment such as Biological Safety Cabinets:

- Type 1 Class A performance Grade 2 filters must be used in all bio-containment applications;
- All filters are mounted as close as possible to the containment facility and the enclosures and ductwork must be of gastight construction;
- Differential pressure testing upstream and downstream of the filter element to monitor pressure drop and identify when element replacement is required;
- Periodic gaseous decontamination of the filter element independent of the containment facility and prior to filter testing;
- HEPA filter shall be tested annually in accordance with AS1807.6 or AS 1807.7 as applicable;
- Prior to testing, filters shall be decontaminated in accordance with AS/NZS 2647;
- Monitor the effectiveness of decontamination with formaldehyde using biological indicators such as spore strips. It is often not possible to wait for 5 days for spore culture strip results to be available; however, the results of tests must be recorded as they provide a record of decontamination over a period of time as well as a confidence check of the technique used. Refer to AS/NZS 2647 Appendix D for details of this process.

Maintenance and replacement of HEPA filter is critical to the containment provided by biological installations and equipment. Consult Section 10.9 of AS/NZS 2243.3:2010 for further information on HEPA filters.

12. HAZARDOUS SUBSTANCE MANAGEMENT

Biological research work can involve the handling and use of hazardous chemical substances that are highly toxic, corrosive or flammable. Inadvertent exposure to chemical agents may cause adverse health effects of an acute or chronic nature or damage the autoimmune system that will, in turn, predispose an individual to infection.

Legislative compliance for work with chemical substances can be achieved by following the UWA procedures and guidelines documented in the Chemical Safety section at the following link:

http://www.safety.uwa.edu.au/topics/chemical

More detailed information may be obtained by consulting AS/NZS 2243.2 Safety in Laboratories - Chemical Aspects or AS/NZS 2243.10 Safety in Laboratories – Storage of Chemicals.

13. USEFUL LINKS

The following links provide a detailed reference source of guidance material on a range of Biosafety topics:

Australian Quarantine and Inspection Service (AQIS)

Belgian Biosafety Server

Biotechnology Australia

CSIRO - Genetechnology in Australia

MSDS for Biological Agents (Health Department, CANADA)

Office of the Gene Technology Regulator (OGTR)

Risk Group - American Biological Safety Association

APPENDIX A

It is not uncommon to find biotechnology research being conducted by individuals who have an engineering background and are unfamiliar with the different groups of microorganisms they may encounter in the course of their work. The following microbes are briefly identified according to group and will provide researchers with a basic foundation of understanding in the identification and containment of infection:

- **Bacteria**

  Bacteria are single cell structures without a nucleus (*prokaryotic domain*). Bacteria display a diversity of morphology (size and shape), but typically have sizes in the range 0.5 – 5 micrometres, while the shape is commonly spherical (*cocci*), rod-shaped (*bacilli*) or spiral (*spirilla*).

  Bacteria replicate by cell division and are prolific on Earth and can be found everywhere; habitats include soil, water, plants, animals and humans. It is estimated that between 500 and 1000 species of bacteria live in the human gut alone. Bacteria are essential for the recycling of nutrients on the planet and without them it would be impossible to maintain the biochemical cycles of our ecosystem.

  The majority of bacteria that exist within the natural flora of the body and those we come into contact with in our daily lives are rendered harmless by the effectiveness of the human immune system. A few species of bacteria are pathogenic and will cause disease in plants, animals and humans; these can be infectious and have vectors of transmission between species.

  Acute or chronic exposure to toxic substances, radiation or other infectious agents may supress the human immune system and make us vulnerable to the naturally occurring bacteria within the body. Bacterial infections are treated with antibiotics; however, abuse and overuse of antibiotics has led to the appearance of methicillin-resistant strains that challenge the current medical resources available to combat disease.

  A further defence against pathogenic bacteria can be achieved by vaccination to confer an artificially acquired immunity to allow the body's natural defence system to resist the infection. In developed countries many bacterial diseases such as *tetanus*, *diphtheria*, *typhoid* and *cholera* have been eradicated through vaccination programmes.

  Examples of common Bacterial Diseases - Anthrax, Botulism, Brucellosis, Cholera, Diphtheria, Gonorrhoea, Legionnaires Disease, Pneumonia, Plague, Salmonella, Septicaemia, Syphilis, Tetanus, Tuberculosis, Typhoid.

- **Viruses**

  Viruses are infectious microorganisms that are smaller than bacteria and can only replicate inside the cells of living organisms. Viruses display a wide diversity of morphology and are typically between 20 and 300 nanometres in size.

  Like bacteria, viruses are prolific in every ecosystem on Earth with many millions of different types occurring. Humans, animals, plants, bacteria and achaea are vulnerable to infection by viruses.

  Viruses do not have a cellular structure like bacteria and do not replicate by division. They resemble organisms since they have genes and evolve by natural selection, but need the mechanism of the host cell in order to create multiple copies of themselves through self-assembly. Viruses have either DNA or RNA genes and are referred to as DNA viruses or RNA viruses according to their genome; however, RNA viruses are more common. Most cell infections by viruses will result in cell death or the cessation of normal cell function, including cell mutations that go on to produce cancer e.g. human papilloma virus.

  Many viruses have a narrow host range and are species specific and may only infect, for example, humans. In each case the vectors of transmission within that population are usually well established. From time to time pandemics occur when viruses will jump species, for example, from animal to human – HIV originates from non-human primates while SARS, a severe respiratory disease in humans, comes from the *coronavirus* that is prevalent in animal populations. The natural host population for such viruses develops some acquired resistance, but it may devastate the new host population.

  Antibiotics are ineffective against viruses and the first line of defence is the body's own immune response system. Because a virus embeds itself within the host cell and uses internal mechanisms to replicate, it is hard to eliminate without damaging the host cell.

  Vaccination is currently the most effective method of preventing virus infection by conferring artificially acquired immunity; however, some viruses such as HIV do not provoke an immune system response and effectively evade the body's defence mechanisms. New generation antiviral drugs are being developed that target the replication process of viruses; these involve placing fake DNA building-blocks into the genome of the virus during replication to render the new virus inactive.

  Examples of common Viral Diseases – Common cold, Dengue fever, Ebola, Encephalitis, Foot and Mouth disease, Hepatitis (A, B, C, D), Hendra, Herpes, HIV, Human Papillomavirus, Influenza, Marburg, Measles, Meningitis, Mumps, Pneumonia, Polio, Rabies, Ross River Fever, Rotavirus, Rubella, SARS, Smallpox, Yellow Fever.

- **Parasites**

  A parasite lives in a close relationship with another organism of a different species in order to live, grow and multiply; in most cases the relationship is detrimental to the host. The range of parasitic relationships may be quite broad, but in biological activities there is usually a large size difference between the parasite and the host. The invasion of a species by bacteria and viruses may also be regarded as a parasitic relationship because the pathogen requires the local organisms to support its life-cycle and replication.

  Ectoparasites, such as mites live on the surface of the host, while endoparasites, such as parasitic worms, exist inside the host - some endoparasites may inhabit the cells of the organism. Parasites, like any invading pathogen, will be subject to the natural defence mechanisms of the host species that will evolve over time to combat attack by parasites, as well as host behavioural changes that may occur. Likewise, parasites evolve and adapt to compete with the host, but have the advantage that their reproduction life cycle is shorter.

  There may be a number of stages associated with the parasites life cycle that involve habitation or colonisation of several species of host. In some cases the parasite may influence the behaviour of the host to facilitate its life cycle. Transmission of ectoparasites may be from plants to animals or humans, while endoparasites require skin penetration, inhalation, ingestion or blood contact to enter a human or animal host.

  In order to complete its life cycle parasites may need to colonise other hosts. Ectoparasites will shed eggs and await contact between the external environment and a new host (fleas and mites); others will transmit via host to host contact. Endoparasites may be shed in faeces or may form larvae inside the body of an animal that is transmitted to a new host when it is digested. This form of transmission can occur from animals in the food chain to humans, for example, sheep infected with *hydatid diseases* that go on to cause cysts to form in the liver and lungs of the host.

  All parasitic infections require a transmission path; this may be simply physical contact or, alternatively, quite complex in the form of transmission vectors that are part of the cycle. In the case of blood-borne parasites the vector may be blood sucking insects such as mosquitos – *malaria* is a typical example of a parasitic disease that causes over half a million deaths annually worldwide.

  Examples of common Parasites – Chagas disease (*trypanosoma cruzi*), Hydatid disease, Lice (body louse, crab louse, head louse), Malaria (*plasmodium malariae*), Morgellon's disease, Primary Amoebic Meningoencephalitis (PAM), Pneumonia, Protozoa (including *plasmodium and trypanosoma*), Scabes, Sleeping Sickness, Trichomoniasis, Worm infections (flukes, hookworm, pinworm roundworm, tapeworm, whipworm).

- **Fungi**

  Fungi belong to the Eukaryotic group of organisms that extends from macro fungi (mushrooms), right down to microorganisms occurring in yeasts and moulds. Fungi are essential to the decomposition and recycling of organic material and nutrient exchange back into the environment. In addition, the metabolites of many species are active ingredients in pharmacology, in particular antibiotics (*penicillins*) and cholesterol lowering drugs (*statins*) as well as many diverse uses in the chemical and agricultural industries. Fungal infections are responsible for some of the most devastating plant diseases in agriculture, and can cause life threatening diseases in humans.

  The fungal kingdom is very diverse and contains many millions of species with only 5% having been scientifically classified. The life cycle of fungi comprises the development of *hyphae*, small knots and branches in the biological material that will clump together to form the *mycelium*, a web-like structure spread throughout the growing environment. When the conditions are right, the mycelium will produce the fruiting body of the fungi that will grow, mature and release spores to propagate the species.

  Spores are of the order of 1 to 40 micrometres in length and can be transported long distances by air currents. Inhalation or body contact with the spores of certain species of fungi will cause disease in humans. Antibiotics are ineffective in the treatment of fungal pathogens because the disease and the medication share the same Eukaryotic cells. In this case the body is totally reliant on its natural defence system for protection against fungal infection.

  Examples of common Fungal Diseases – Aspergillosis, Athlete's Foot (*tinea*), Blastomycosis, Candidiasis, Coccidioidomycosis, Cryptococcosis, Ergot, Fungal Endocarditis, Pyrenophora, Ringworm (*tinea*), Sporotrichosis, Tinea.

- **Prions**

  Prions are infectious pathogens that do not contain nucleic acid (DNA or RNA genes). This is quite different from the four infectious pathogen groups discussed previously that all have DNA, RNA or both. The proteins from which prions are made are found in the bodies of healthy animals and humans, but existing in the correct folded form; a prion is an infectious pathogen comprising a protein in a misfolded form.

  Prions are responsible for Transmissible Spongiform Encephalopathies in mammals. This fatal, untreatable disease occurs as *Scrapie* in sheep and *Bovine Spongiform Encephalopathy* (BSE or Mad Cow Disease) in cattle. The transmission form to humans is *Creutzfeld-Jakob Disease* (CJD) and occurs as the result of contact with infected animal tissue.

Prion infection occurs when a protein in the misfolded form enters the organism and becomes a template to convert healthy proteins into disease prions. After an incubation period, this infection rapidly progresses as a cumulative process that destroys healthy brain tissue, causing spongy holes to appear and severing neural connectivity through the build-up of amyloid plaques. The structural stability of the altered proteins means that the new structure is resistant to denaturation (changes in structure) making disposal and containment very difficult.

Eight proteins demonstrating prion-like behaviour have been found in different fungi species, but so far there is no evidence that they cause disease. Different isoforms of protein type exist; PrP has the same structure of protein that prions originate from and occurs in the un-diseased state. PrP^{C} (c designates cellular) is the normal protein found in the membrane of cells and PrP^{SC} (sc designates *Scrapie*) is the disease protein isoform of PrP.

As a result of the relatively recent discovery of prion agents, knowledge is still developing in this area. There is currently no treatment for the condition and little in the way of diagnosis, or detection of infection. Because of the structural stability of prions, sterilisation and other common denaturation methods are not effective in deactivating prions. If the prion tertiary structure is not completely destroyed, it is still possible for the prion to be renatured back to an infective state.

Examples of common Prion Diseases – Alpers Syndrome, Bovine Spongiform Encephalopathy (BSE), Chronic Wasting Disease (CWD), Creutzfeld-Jakob Disease (CJD), Fatal Familial Insomnia (FFI), Gerstmann-Straussler-Scheinker Syndrome (GSS), Kuru, Scrapie, Transmissible Mink Encephalopathy (TME).