Introduction to Microbiological Biosafety

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Microorganisms and Biological Hazards

For the purposes of biosafety, a **microorganism** is defined within Australian/New Zealand Standard 2243.3:2022 'Safety in Laboratories Part 3: Microbiological Safety and Containment' (AS/NZS 2243.3) as a microscopic organism, and may include bacteria, viruses, fungi, parasites, protozoa, unicellular algae, viroids and prions.

A **biological hazard** is defined as any potential microbiological source of harm. Therefore, in the laboratory, any sample that may harbour or support the growth of microorganisms is considered a potential biohazard. This includes environmental samples (e.g., soil, water, wastewater), clinical specimens (e.g., blood, tissues, bodily fluids, and faeces), and cells and cell lines.

Microorganisms vary in their ability to cause infection, and in the severity of outcomes associated with infection. Apart from obvious risks to human health, microorganisms can be associated with disease in animals and plants that can cause serious harm to the economy or the environment. Some microorganisms may also present a higher risk to specific personnel in the laboratory. For example, those who are immunocompromised, diabetic, pregnant, or breastfeeding may have an increased risk of infection or of serious illness or negative outcomes associated with infection.

All work with viable microorganisms and biologically hazardous samples requires the use of aseptic techniques and risk controls to minimise the risk to people and the environment. These techniques are good laboratory practice that also help to prevent contamination of samples and maintain scientific integrity.

For biological samples with an unknown microbiological profile, the basic approach is to regard them as potentially containing pathogens and to handle them following standard precautions.

Where there is a known or high likelihood of hazard (e.g., a blood sample derived from a patient with acute HIV infection, or work with an isolated risk group 2 bacteria), a documented risk assessment is required to identify additional controls that are required to address the risk. Work with these samples must only be done in a microbiological containment facility. The microbiological risk group classification and information about the mode of transmission and the nature of the work being performed guide the risk controls that are required.

Standard Precautions

Standard precautions are the basic level of risk controls that apply to all work with microorganisms and biological hazards. These principles provide a basic level of protection for laboratory workers handling biological samples. Work involving known or suspected risk group 2 or higher microorganisms will require additional controls.

Standard precautions involve:

- Appropriate hand hygiene – washing hands with soap and water or using a disinfectant hand rub after you finish work and before you leave the lab.
- Using appropriate personal protective equipment – a long-sleeved laboratory gown, enclosed footwear, and gloves at minimum.
- The safe use and disposal of sharps.
- Routine decontamination of work surfaces with disinfectant – after each work session and after any spill or splash.
- Decontamination of reusable labware and equipment that has been in direct contact with biological samples.
- Maintaining a clean laboratory that is free of dust that may harbour or transmit microorganisms.
- Aseptic technique, good microbiological practice and prevention of cross-contamination.
- Decontamination of waste – e.g., autoclaving, chemical disinfection, disposal via biohazard waste stream for incineration.
- Cleaning up biological spills following spills management procedures.
- Undertaking aerosol-generating procedures in a biological safety cabinet.
- Do not use a laminar flow for work with viable microorganisms or samples known or reasonably expected to contain viable microorganisms.
- Always consider personnel safety, follow SOPs, properly use safety equipment and receive training where necessary.

Microbiological Risk Groups

To support the appropriate management of risk, microorganisms are categorised into different ‘risk groups’ based on their ability to cause harm to people, animals, and the environment. There are four

tiers of risk groups (numbered 1 to 4), with 1 being the lowest level of risk and four presenting the highest risk.

In Australia, these risk groups are defined in AS/NZS 2243.3:2022. The definitions cover microorganisms that are infectious to humans, terrestrial and aquatic animals and plants, and those that are carried by invertebrate vectors.

The Standard also provides tables with *examples* of microorganisms categorised by risk group and type. Where a microorganism does not appear in the tables, or where the strain you are using has unique properties (e.g., toxin production or multi-drug resistance), an assessment must be undertaken to determine the appropriate risk group and associated containment level based on the criteria for risk groups defined within the Standard (see below).

Risk Group 1	**Low individual and community risk** Unlikely to cause human, animal, plant, or insect disease. Low risk to industry, the environment, and the community. Already widely distributed within Australia. E.g. *Lactobacillus acidophilus*, E. coli K-12, *Saccharomyces cerevisiae,* bacteriophage λ Generally suitable for work in Physical Containment Level 1 microbiological containment facilities
Risk Group 2	**Moderate individual risk, limited community risk** Can cause human, animal or plant disease, but is unlikely to be a significant risk to the community, livestock, or the environment. Laboratory exposures may cause infection, but effective treatment and preventative measures are available, and the risk of spread is limited. For plant pathogens and microorganisms carried by insects, may include those that are exotic to Australia, but with a limited ability to spread and can be easily controlled. E.g., *Acinetobacter* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Toxoplasma gondii*, *Candida albicans*, Adenovirus, Dengue virus, Human respiratory syncytial virus, Citrus canker (*Xanthomonas axonopodis*), Abalone herpesvirus Generally suitable for work in Physical Containment Level 2 microbiological containment facilities
Risk Group 3	**High individual risk, limited to moderate community risk** Usually causes serious human, animal or plant disease and may present a significant risk to laboratory workers. It may present a limited to moderate risk if spread in the community or the environment, but there are usually effective preventative or treatment measures available. For plants, this would include those that are a high risk to industry or a region within Australia, and any microorganism that is exotic and readily spread naturally without a vector.

	For infectious microorganisms carried by invertebrates, this risk group includes ones carried by insects that can easily spread. E.g., *Bacillus anthracis*, *Brucella* spp., *Coxiella burnetii*, *Rickettsia* spp., Avian influenza virus, Newcastle Disease Virus, Japanese encephalitis virus (excluding Nakayama strain), Human T-lymphotrophic virus type 1 and 2, grapevine rust (*Phakopsora euvitis*). Generally suitable for work in Physical Containment Level 3 microbiological containment facilities
Risk Group 4	**High individual and community risk** Usually produces life-threatening human or animal disease and represents a significant risk to laboratory workers. May be readily transmissible from one individual to another in the community. Effective treatment and preventative measures are not usually available. For microorganisms transmitted by invertebrates, also includes microorganisms that are very high risk to the environment that are exotic and able to readily disperse or are carried by invertebrates that are difficult to visually detect. E.g., Ebola virus, Hendra virus, Lassa virus, Marburg virus, Tick-borne encephalitis virus Can only be handled in Physical Containment Level 4 microbiological containment facilities

A microbiological physical containment facility is required for all work involving viable risk group 2 (or higher) microorganisms or samples known to contain these. A documented risk assessment is required for all microorganisms of risk group 2 (or greater) to determine if the work requires additional controls, precautions, or a different containment level.

Please note that many other countries use risk group classification for microorganisms, but there is no internationally agreed classification. Therefore, a microorganism that is classified as risk group 2 in America, for example, may not automatically meet the requirements for risk group 2 classification in Australia. This is particularly the case where a microorganism is not endemic within Australia.

What requirements apply?

If you are working with microorganisms or samples known or reasonably suspected to contain risk group 2 (or higher) microorganisms, then the requirements of AS/NZS 2243.3:2022 apply. Staff and students are required to comply with the conditions of this standard to meet their obligations under the Work Health and Safety Act and Regulations.

Biological Risk Assessment

A biological risk assessment must be completed for all research and teaching activities involving the use of risk group 2 (or higher) microorganisms or samples that are known to contain these.

Biological risk assessments specifically take into account the nature of the microorganism(s) that are being handled, combined with the risks associated with the handling method(s). This type of risk assessment considers:

- Hazards associated with an infectious microorganism or biohazardous sample, e.g.:
  - Pathogenicity, virulence, communicability, invasiveness
  - Infectious dose
  - Origin of material being handled
  - Resistance to antibiotics, disinfectants
  - Availability of vaccines, prophylactic interventions
  - Endemic or exotic nature of the microorganism(s)
  - Zoonotic potential
- The transmission pathways of the microorganism
- Activities that may cause exposure or infection
- Activities and factors that may lead to loss of containment
- The training, competencies, and experience of laboratory personnel

A biological risk assessment form has been developed and must be completed and submitted to the Biosafety Committee for review and approval prior to commencement of the following types of research:

- Work with viable risk group 2 microorganisms, including bacteria, viruses, parasites, fungi, algae, or prions, that are pathogenic, allergenic, or toxic to humans, animals, plants, or aquatic organisms.
- Work with unfixed human or animal bodily fluids or tissues where the samples are derived from humans or animals known or reasonably expected to be infected with a risk group 2 (or higher) microorganism.
- Work with plants knowingly infected with a risk group 2 (or higher) microorganism.
- Work with primary cells or cell lines that are known or reasonably expected to contain risk group 2 (or higher) microorganisms.
- Isolation, culture or enrichment of unknown microorganisms from environmental samples where the methods used are designed or intended to isolate, culture or enrich risk group 2 (or higher) microorganisms.
- Work involving Security Sensitive Biological Agents – please contact the Research Compliance Officer: Biosafety before preparing an application.
- Work with or storage of viable or non-viable risk group 3 or risk group 4 microorganisms (or samples containing these) - please contact the Research Compliance Officer: Biosafety before preparing an application.

Microbiological containment facilities

Research with microorganisms and samples known or reasonably expected to contain risk group 2 (or higher) microorganisms must be undertaken within a microbiological containment facility of the appropriate class (e.g., laboratory, animal facility, plant facility) and level (i.e., PC1, PC2, PC3, PC4).

The requirements for microbiological containment facilities are described in AS/NZS 2243.3:2022.

At The University of Adelaide, the Research Compliance Officer: Biosafety undertakes inspections to confirm compliance of facilities with AS/NZS 2243.3:2022. A register of compliant and approved facilities is maintained by the Institutional Biosafety Committee.

Further information about certification, requirements and use of microbiological containment facilities is available on the Containment Facilities website.

Biosafety Committee

The University of Adelaide Biosafety Committee operates as a function of the Institutional Biosafety Committee (IBC). The responsibilities of the committee are to provide training materials, monitoring and surveillance, review of biological risk assessments, facility inspections and audits and record keeping for microbiological research and teaching activities. The scope of the Committee's responsibilities is limited to University of Adelaide staff and title holders and to external parties using University of Adelaide facilities.