ASSIST
AUSTRALIAN SCHOOL SCIENCE INFORMATION SUPPORT FOR TEACHERS AND TECHNICIANS

GUIDELINES for best practice for microbiology in Australian schools

These guidelines were produced by the Science ASSIST project managed by the Australian Science Teachers Association (ASTA) in consultation with the Science Education Technicians Association (SETA). The Science ASSIST project is supported by the Australian Government Department of Education and Training through the Mathematics and Science Participation Program.

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Preface

Microbiology is an exciting and evolving area of science with links to many sections of the Australian Curriculum: Science. It is the study of biological organisms that are impossible to see individually with the naked eye.

Safety issues are a significant consideration as the subject is practical-based and there is potential for exposure to infectious organisms. It is the strict observance of correct procedures, which enables staff and students to work safely with microorganisms.

Australian schools are situated in geographical locations subject to a wide variety of local jurisdictional regulations as well as climatic conditions. They may be a part of a state/territory school jurisdiction that has strict guidelines for microbiology, or one that allows schools to make their own decisions based upon a risk management approach to activities conducted. Alternatively, a school may be part of a non-government sector or an Independent school that is responsible for developing its own policies and procedures.

These guidelines outline the underpinning knowledge and laboratory techniques required for schools to successfully prepare, deliver and disassemble microbiology practical activities.

Acknowledgements

The Australian Standard AS/NZS 2243.3.2010 *Safety in laboratories Part 3: Microbiological safety and containment* and the following related overseas documents, form the basis for developing this document.

- American Society for Microbiology. 2012. *Guidelines for Biosafety in Teaching Laboratories.* American Society for Microbiology website, https://www.asm.org/images/asm_biosafety_guidelines-FINAL.pdf
- American Society for Microbiology. 2012. *Appendix to the Guidelines for Biosafety in Teaching Laboratories.* American Society for Microbiology website, http://www.asm.org/images/Education/FINAL_Biosafety_Guidelines_Appendix_Only.pdf
- Centers for Disease Control and Prevention. 2009. *Biosafety in microbiological and biomedical laboratories (BMBL)* 5th Edition, Centers for Disease Control and Prevention website, http://www.cdc.gov/biosafety/publications/bmbl5/
- Microbiology in Schools Advisory Committee (MISAC). 2010. *Suitable and unsuitable micro-organisms,* MISAC website, http://www.misac.org.uk/PDFs/MiSAC_suitable_and_unsuitable_micro-organisms.pdf
- Society for General Microbiology. 2006. *Basic Practical Microbiology – A Manual.* Microbiology Online website, http://www.microbiologyonline.org.uk/file/ca2189fba3b39d24c5a44c1285d0082c.pdf
- Society for General Microbiology. 2008. *Practical Microbiology for Secondary Schools.* Society for General Microbiology, Microbiology Online website, http://microbiologyonline.org/file/a53ae1e6c21fd83d14325f7f81dc2fc2.pdf
- World Health Organization. 2004. *Laboratory biosafety manual.* Third edition. World Health Organization website, http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

(All documents were accessed in December 2016.)

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Contents

Chapter 1 Classification of microorganisms

Microbiology is the study of biological organisms that are impossible to see individually with the naked eye. Students should study microbiology because it has many applications in society, including in:

- the food industry.
- diagnostic microbiology—the study of disease in animal and plant species.
- the pharmaceutical industry—production of antibiotics and vaccines to treat disease.
- the biotechnology industry.

1.1 Beneficial uses of microorganisms

Some microorganisms are used to benefit the lives of humans and animals in areas such as food production, health and understanding disease. Some examples of beneficial microorganism use are:

Food production

- Yeast is used in the manufacture of beer and wine.
- Lactic acid bacteria are used to make fermented milk products such as yoghurt, sour cream, and buttermilk.

Medical

- Production of antibiotics and vaccines.
- Microorganisms used in biotechnology play a central role in recombinant DNA technology and genetic engineering.

Other associations

- The microbes that normally live in association with humans on the various surfaces of the body are called ‘normal flora’. These microbes are known to protect their hosts from infections and otherwise promote good health.
- Microbes help purify wastewater in wastewater treatment facilities.
- Microbes help reduce atmospheric nitrogen and transform it to ammonia important for agriculture.[1]

1.2 Microorganisms detrimental to human health and environment

Many microorganisms are pathogenic, which means they are capable of causing disease.

Common examples of pathogenic bacteria include:

- *Salmonella*—causes food poisoning in humans typically through the ingestion of undercooked chicken or poor food hygiene practices.[2]
- *Legionella*—occurs naturally in soil and in untreated potting mix and grow well in water situated in cooling towers of air conditioning units and hot tubs[3]. It is potentially deadly to humans.
- *Staphylococcus aureus*—causes most staphylococcal infections such as skin infections, pneumonia, food poisoning, toxic shock syndrome and blood poisoning (bacteraemia).[4]

Some fungi can be pathogenic to some humans, for example, infectious agents such as the common mould *Aspergillus*. Whilst most people can breathe in these spores without effect, they can cause allergies, asthma and infections in people with weakened immune systems or lung disease.[5] *Candida* is a yeast-like fungus that occurs commonly as part of the normal flora

of the mouth, skin, intestinal tract, and vagina, but can cause a variety of infections. *Candida albicans* is the usual pathogen in humans.[6]

A very small number of species of Protozoa cause disease in people, for example, *Plasmodium species*, which cause malaria.[7]

When preparing to study microbiology, it is important to understand the different types of microorganisms, the hazards associated with them and the risk management strategies required.

1.3 Types of microorganisms

Microorganisms are very diverse and include the major groups of bacteria, fungi (moulds and yeasts), algae, protozoa and viruses. Many microorganisms are made up of only a single living cell (unicellular), some are multicellular and others do not have a true cellular appearance (acellular – viruses). When bacterial or yeast cells grow and multiply on agar plates they form colonies. These colonies can contain millions of cells.

Microorganisms are living cells and the main types studied in school microbiology include bacteria, fungi, and protozoa.

1.3.1 Bacteria

Bacteria are prokaryotic single celled organisms. They have a rigid cell wall, no nuclear membrane, no organelles in the cytoplasm, and have genetic material in the form of single continuous strands forming coils or loops. These are characteristic of all organisms in the kingdom Monera, including bacteria and blue-green algae.[8]

Typically, 1–2 micrometres in length, bacteria have a number of shapes, ranging from cocci (circular shape) to rods and spirals.

Bacteria can be found everywhere: on the palm of our hands, in our digestive system, on a laboratory bench, in the atmosphere. Some species of bacteria enable humans and animals to digest food, fight disease and protect from more debilitating diseases. Other bacteria will cause disease, which is often easily transmissible to others humans or animals.

1.3.2 Fungi

Fungi are eukaryotic unicellular or multicellular organisms. They have as their fundamental structural unit a cell type that contains a rigid cell wall, specialised organelles in the cytoplasm, a membrane-bound nucleus enclosing genetic material organised into chromosomes, no chlorophyll and an elaborate system of division by mitosis or meiosis, characteristic of all life forms except bacteria, blue-green algae, and other primitive microorganisms.[9]

Fungi include microorganisms such as yeasts and moulds. Some fungi are pathogenic to humans, and most are pathogenic to plants.[10]

Fungi have an essential role in the decomposition of dead and rotting soil and organic matter, and can also be found on foods in our kitchens. They have beneficial uses such as the production of antibiotics, as a leavening agent in bread production and as a direct food source such as mushrooms.

1.3.3 Protozoa

Protozoa are larger than bacteria, generally 10–52 micrometres, are unicellular eukaryotic microorganisms that lack a rigid cell wall. They have been found in almost every kind of soil

environment from peat bogs to arid desert sands. They teem in the deep sea as well as near the surface of waters, and can be found even in frigid Arctic and Antarctic waters.

Some species of protozoa are part of the normal microbial flora of animals, and live in the guts of insects and mammals, helping to break down complex food particles into simpler molecules. Others are parasitic, causing human diseases such as malaria, sleeping sickness and dysentery.

1.4 Classification of microorganisms by risk group

The World Health Organisation (WHO) has produced an extensive document on biosafety and has recommended that countries draw up classification of microorganisms within their boundaries according to the degree of risk considering:

1. pathogenicity of the agent
2. the mode of transmission and host range of the organism
3. local availability of effective preventative measures
4. local availability of effective treatment.[11]

The Australian Standard AS/NZS 2243.3.2010 *Safety in laboratories Part 3: Microbiological safety and containment* has drawn up the following classification for microorganisms that are infectious for humans by risk group.

1.4.1 Risk Group 1

Risk Group 1 (RG1) includes those microorganisms that are unlikely to cause human or animal disease in healthy adult humans.

The AS/NZS 2243.3:2010 *Safety in Laboratories – Microbiological safety and containment* does not list Risk Group 1 microorganisms, as there are too many. Some examples used in Australian schools are *Escherichia coli (K-12)*, *Staphylococcus epidermidis, Micrococcus luteus, Bacillus subtilis, Saccharomyces cerevisiae* and *Penicillium chrysogenum*.

1.4.2 Risk Group 2

Risk Group 2 (RG2) includes those microorganisms that are unlikely to pose a significant risk to laboratory workers, 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.

Examples of microbes that are classified risk group 2 include: *Bordetella pertussis* (causative agent of whooping cough), *Chlamydia spp.* (a common sexually transmitted disease) and *Vibrio cholerae* (cause of epidemic Cholera).

1.4.3 Risk Group 3

Risk Group 3 (RG3) includes those microorganisms that usually cause serious human or animal disease and may present a significant risk to laboratory workers. They could present a limited to moderate risk if spread in the community or the environment, but there are usually effective preventative measures or treatments available.

Examples of microbes that are classified Risk Group 3 include: *Bacillus anthracis* (the etiological agent of Anthrax), *Coxiella burnetii* (the causative agent of Q fever) and *Yersinia pestis* (the causative agent of plague).

1.4.4 Risk Group 4

Risk Group 4 (RG4) includes those microorganisms that usually produce life-threatening human or animal disease, represent 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.[12]

Examples of microbes that are classified risk group 4 include: Ebola virus (cause of haemorrhagic fever) and Lassa virus (cause of Lassa haemorrhagic fever).

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Chapter 2 Classification of laboratories

2.1 Working with microorganisms—Physical containment levels

Microorganisms vary in their capacity to infect humans, however, it is considered good practice to consider all microorganisms as potential pathogens and use a combination of strategies to handle them safely. These include

- structures such as building designs
- engineering functions such as air pressure controls
- specialist equipment such as biological safety cabinets
- standard microbiological techniques.

Physical containment is the term used to describe procedures and structures designed to reduce or prevent the release of viable organisms into the outside environment.[13] In Australia, laboratories are classified into four levels of Physical Containment (PC 1–4). The level of containment must be at least the level appropriate for the risk group of the microorganism being used in the laboratory i.e. PC1 for RG1, PC2 for RG2 etc.

2.1.1 Physical Containment Level 1 (PC1)

A Physical Containment Level 1 laboratory is suitable for work with material likely to contain microorganisms that are classified as Risk Group 1 microorganisms. They require no special containment equipment and are suitable for schools and undergraduate teaching laboratories. Work may be carried out on the open bench as long as hazard levels are low and standard microbiological techniques are followed in order to protect laboratory personnel. Specimens that have been fixed or inactivated may be handled in a PC1 facility.[14]

2.1.2 Physical Containment Level 2 (PC2)

A Physical Containment Level 2 laboratory is suitable for work with 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 must be used.

2.1.3 Physical Containment Level 3 (PC3)

A Physical Containment Level 3 laboratory is suitable for work 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.

2.1.4 Physical Containment Level 4 (PC4)

A Physical Containment Level 4 laboratory is suitable for work with microorganisms classified as Risk Group 4 microorganisms. A PC4 laboratory or facility is situated in a building separate from other laboratories facilities or constructed as an isolated area within a building.[15]

2.2 Australian school facilities

Australian school science laboratories are generally constructed to Physical Containment 1 (PC1), the most basic level. At this level, the laboratory is suitable for work with microorganisms where the hazard levels are low, and where laboratory or facility personnel can be adequately protected by standard microbiological techniques.[16] Standard

microbiological technique is where staff and students are proficient in methods to contain any uncontrolled spread of microbes in order to protect:

- practical investigations from becoming contaminated with microbes from external sources
- the operators (students, teachers and technicians) from the very small possibility of infection.[17]

Laboratory design considerations for work with Risk Group 1 microorganisms in PC1 labs.

- Laboratory floors, benches and seating must be made from smooth impervious material to ensure they are easily cleanable, easily decontaminated and resistant to damage by the cleaning agents and/or disinfectants that will be used in the laboratory.

  Note: Laboratories that have carpet covering any part of the floor is not deemed to be suitable as a PC1 laboratory. Carpets can harbour fungal spores and bacteria, even when an activity is taking place several metres away.

- Bench top surfaces must also be made of heat resistant material.
- Sink for hand washing with potable hot and cold water services shall be provided inside the laboratory near the exit.
- Designated storage or hanging facilities for protective clothing must be available within the facility.
- Open spaces between and under benches, cabinets and equipment shall be accessible for cleaning.
- Internal fittings and fixtures must be arranged to minimise dust accumulation.
- Provision of an eyewash and safety shower area.

Schools wishing to conduct activities using RG1 microorganisms should assess their own facility for compliance with the requirements of AS/NZS 2243.3 (Section 2.5)

If school facilities allow, a specialised microbiology laboratory could be considered for microbiology activities. Select a laboratory that is easily accessible from the preparation room, has facilities at Physical Containment 1 level, and have little or no through foot traffic. Equipment such as an incubator may be stored and used in this laboratory if it is not likely to be interfered with by other students.

However, it is safe to use the same room as a microbiology laboratory and a teaching room for other classes. When not in use and whilst non-microbiology classes are in session, all cultures should be stored properly (e.g., in incubators or refrigerators) and not left out in common areas. Cultures and waste should be decontaminated immediately or stored in a separate, non-shared location that is not accessible by students. Laboratory benches should be disinfected immediately prior to and following microbiological activities to prevent contamination of microbiology work and subsequent student activities.

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Chapter 3 Microbiology in Australian schools

Australian school jurisdictions permit the use of microorganisms in Risk Group 1 where there is a low risk of disease to students, teachers and laboratory technicians. The use of Risk Group 1 microbes in schools does not negate the importance of good microbiological practice.

It should be noted that even though microorganisms are from RG1, some could still pose a low level of risk to the community, as they can be capable of causing disease if provided with appropriate conditions (referred to as opportunistic). People who are immunocompromised or immunosuppressed are at greater risk, and along with those who are pregnant or may become pregnant, should seek advice from their medical professional before embarking on microbiological activities.

It is good practice to regard all microorganisms, regardless of their risk group, as potential pathogens and to handle them with standard microbiological techniques to minimise risk to laboratory staff, students and the environment.

Microbiological activities can be safely undertaken in schools, provided proper facilities are in place and precautions are identified and implemented. These include:

- appropriate risk assessment processes
- consideration for the microorganisms to be used (see Chapter 1)
- correct laboratory facilities (see Chapter 2)
- standard microbiological techniques (see Chapter 4).

3.1 Risk assessment

It is a legal requirement for every workplace to manage risks to health and safety so far as is reasonably practicable. It is a continuous process of identifying hazards, assessing and controlling risks that may affect the functioning of a workplace.[18]

A biological risk assessment of a school microbiology activity needs to ensure that the risks are not underestimated or there could be undesirable consequences. Living cells have the capacity to multiply in optimum conditions and all microorganisms should be considered as if they are pathogens. Incorrect handling of microorganisms can cause laboratory-acquired infections. Therefore, it is vital that due consideration is given to the type of microorganism being used as well as the procedures being implemented. A risk assessment should be used to alert staff and students to the hazards of working with infectious agents and for the need for developing proficiency in the use of selected safe practices and equipment.

According to *Biosafety in microbiological and biomedical laboratories* (BMBL)[19], the following five steps should be considered:

1. **Identify agent hazards and perform an initial assessment of risk**

   List all known microbes used, the hazardous characteristics of a known infectious or potentially infectious agent or material and the likely route of transmission (via skin, eyes, hand to mouth, nasal inhalation etc.), consider if any wild or unknown microbes will be introduced. Also, consider the susceptibility of certain individuals in the laboratory to infection from the microorganism.

2. **Identify laboratory procedure hazards**

   List all procedural hazards that can result in a person's exposure to an agent, including equipment used, the complexity of the task and the laboratory environment, including decontamination processes.

3. **Make a determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment**

   Determine control strategies by considering the laboratory facilities, techniques and processes used to prevent exposure.

4. **Evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment**

   Evaluate the technical proficiency of staff and students—their ability to control hazards, the presence of good microbiological habits and implement control measures, eliminate, substitute, engineering controls, administrative control, personal protective equipment (PPE). Also, consider the behaviour of the students.

5. **Review the risk assessment with a biosafety professional, subject matter expert, and the IBC.**

   Review the risk assessment with a person knowledgeable in biosafety practices before work proceeds. After the activity, monitor, review and document the effectiveness of the control measures.

Before schools embark on working with microorganisms they should ask the following questions and perform a site-specific biological risk assessment.

- What microorganism is being used? Is it a Risk Group 1 microorganism?
- Do the school facilities comply with the requirements of Physical Containment Level 1 laboratories?
- Does the school have the necessary equipment for sterilisation and decontamination procedures?
- Does the staff have training in microbiological skills?
- What manipulations are being performed with the microorganism? Are methods being used to eliminate or minimise exposure to potentially infectious material via aerosols, splashes, ingestion, absorption and accidental inoculation?
- Are any staff or students wishing to participate in microbiological activities immunocompromised or immunosuppressed (include those who are pregnant or may become pregnant, or are living with or caring for an immunocompromised individual)? These individuals are more prone to infections and they should consult a doctor to determine whether their participation is appropriate.

The AS/NZS 2243.3:2010 *Safety in Laboratories – Microbiological safety and containment* states:

‘*2.6.5 At-risk persons*

> *Persons who are immunosuppressed, immunocompromised, or otherwise unduly vulnerable to infection, such as persons who are diabetic, should inform their supervisor or person responsible for microbiological safety of their condition so that appropriate action may be taken. Some microorganisms that are regarded as part of the normal flora of humans or animals may be pathogenic for immunocompromised persons.’*

3.2 School work levels

Three UK organisations: Society for General Microbiology (SGM), Microbiology in schools advisory committee (MISAC) and CLEAPSS, have categorised microbiology activities conducted in schools into three levels.

Science ASSIST has adapted these levels, by subdividing the UK level 2 to create an additional level for application to the Australian context.

Note: These four levels are not the same as levels of containment, but guidelines for suitable activities conducted in schools. Each higher level includes the microorganisms used in lower levels.

Safety Note: All microbiology work with students, requires close and constant supervision at each level

Level 1 [Very low risk] Observation and growth of certain microorganisms

- **Microorganisms:** which have little, if any, known risk.
  - **Limited to algae, yeasts, moulds and bacteria used for food purposes**, some moulds and commonly occurring bacteria where they grow naturally on decaying vegetable material e.g. brewer's or baker's yeast and certain protozoa or moulds, such as bread mould.
  - These microorganisms to be cultured on the substances on which they grow naturally in closed containers at the ambient room temperature, except for culturing yogurt at 43°C
  - No microorganisms associated with meat or fish.
  - No agar plates
- **Staff training:** No specialist training required for teacher or technician.
- **Facilities:** No special facilities required or need for a steriliser.
- **Waste:** No specialist treatment: Able to be placed into the regular waste or put down the sink.

Level 2 [Low risk]: Simple environmental sampling

- **Microorganisms**: sourced from the environment where the risk of procuring human pathogens is unlikely.
  - Cultured only in closed containers on nutrient agar plates or broth at a maximum of 30°C. The only exception is the sampling of airborne particles for the Pasteur broth activity
  - No subculturing of cultures.
  - Nutrient or plain agar/broth to be used where appropriate.
- **Staff training:** Staff to be trained in sterilisation and decontamination procedures.
- **Facilities:** PC1 facilities required. Autoclave or pressure cooker required for sterile preparation of agar/broth etc. and for decontamination.
- **Waste:** to be sterilised before disposal.

Level 3 [Low-medium risk]: The growth and subculture of pure cultures of microorganisms

Note: schools should check whether level 3 activities are permitted in their school jurisdiction, as certain subculturing is prohibited in some jurisdictions

- **Microorganisms** sourced from school biological suppliers and limited to
  - Certain protozoa (including slime moulds, e.g. *Physarum polycephalum*)
  - Risk Group 1 Fungi and bacteria (Low risk—not associated with disease in healthy adult humans)
  - Risk assessment to consider immunosuppressed staff and students.
  - No subculturing from plates or broths inoculated by students.
- **Staff training:** Staff to be highly trained

- in aseptic technique and recognise contamination of pure cultures.
- to subculture pure samples obtained from reputable suppliers for student use.
- Additional staff recommended for student supervision.

- **Facilities:** PC1 facilities required. Autoclave or pressure cooker required for sterile prep of agar/broth etc. and for decontamination.
- **Waste:** to be sterilised before disposal.

Level 4 [Medium-high risk]: Advanced work in subculturing and manipulations

This level is beyond the scope of this document

It is expected that microorganisms, facilities and waste guidelines will be similar to Level 3 with staff highly trained in microbiology and the manipulations required

3.3 Choice of microorganism

Science ASSIST recommends that schools choose the microorganism with the lowest level of risk that will meet the learning outcomes. Schools need to ensure that they have the required facilities and equipment and the necessary staff training to be able to manage the risks.

When considering microorganisms for Level 3 work, table 1 suggests a number of Risk group 1 microorganisms that are readily available from reputable Australian suppliers. See Science ASSIST *School science suppliers list* https://assist.asta.edu.au/resource/664/school-science-suppliers. These microorganisms are suitable for use in the activities included in this guideline.

Table 1: Risk group 1 microorganisms suitable for use in Australian schools

Microorganism	Gram stain	Shape	Description	Habitat
Bacillus subtilis	positive	Bacillus (rod shaped)	4-10µm long and ~0.25-1µm diameter Singles or in chains	soil air, water, animals
Escherichia coli K-12 strain*	negative	Bacillus (rod shaped)	~ 2µm long and ~0.25-1µm diameter Single cells	gastro intestinal tract of humans and animals
Micrococcus luteus	positive	Coccus (spherical)	1.0-1.8µm diameter irregular clusters, tetrads or pairs	mammalian skin, soil
Mucor – positive and negative strains	n.a.	Hyphae, sporangiophores, some branched ending in round sporangia, sporangiospores	filamentous (long thread-like structure)	soil, plants, decaying fruit and vegetable matter
Penicillium chrysogenum **	n.a.	Hyphae, branched conidiophores, conidiospores	filamentous (long thread-like structure)	blue-green mould on stale bread, fruit and nuts. Used in production of green and blue mould cheese

Physarum polycephalum	n.a.	Slime mould	bright yellow mass of multinucleate protoplasm	under the bark of decaying trees and amongst leaf litter on the forest floor
Saccharomyces cerevisiae	n.a.	Yeast: oval shaped cells with budding	globular shaped green yellow	gastrointestinal tract, body surfaces of insects and warm-blooded animals. Used in fermentation of food.
Staphylococcus epidermidis	positive	Coccus (spherical)	0.5–1.5µm diameter cocci that usually form in clusters	skin, mucous membranes

* *E. coli* K12 strain is considered a low risk of infection due to absence of virulence genes [20]

**"*Penicillium chrysogenum* is considered suitable for use in schools however, it is important to consider that this strain may produce a large number of spores that cause allergies and asthma attacks in some users. This fungus should be handled before sporulation occurs.[21] Further information can be found at http://www.misac.org.uk/PDFs/MiSAC_suitable_and_unsuitable_micro-organisms.pdf "

3.4 Science ASSIST student practical activities and SOPs

These SOPs are located in Appendix 1.

3.4.1 Student activities suitable for level 1:

SOP: Examining life in pond water

Life in pond water consists of small animals that can be viewed macroscopically or microscopically. Different samples of water consist of differing animals from different habitats. This activity looks at how living things satisfy their needs for food, water and air.

SOP: Fermentation of yeast

Yeast requires specific conditions in which to ferment and become active. When used in food production particularly bread making, the yeast must be at a certain temperature to grow and make the bread rise. This activity examines the fermentation of yeast and asks ‘What is the best temperature for yeast to grow?’.

SOP: Making yoghurt

Yoghurt is a fun way to look at the use of microbes in food production.

SOP: Examine mushroom spores

Fungal spores are microscopic. However, we can see the pattern they make on paper when they are released from the gills of the mushroom by making a spore print.

SOP: Growing fungi on bread

Bread mould is a simple fungus, which derives its food from a variety of materials such as grains, fruits, vegetables or flesh. Mould spores are tiny and usually remain suspended in air. As soon as it finds the right environment for it to grow, the spores transform into the living fungus.

3.4.2 Student activities suitable for level 2

SOP: Microbes are everywhere

This is a microbiological activity to gain techniques in environmental sampling and to look at microbes in the environment around us.

SOP: Pasteur's experiment

An investigation to demonstrate Pasteur's experiment that microbial life does not spontaneously generate in sterile nutrient broth.

SOP: Preparing agar plates

A step-by-step guide to preparing and sterilising nutrient and plain agar for aseptic preparation of agar plates and broths. The science technician usually prepares agar plates, however this is also a suitable activity for students.

3.4.3 Student activities suitable for level 3:

SOP: Physarum polycephalum care and use

Physarum polycephalum is a slime mould that is easily demonstrated to show growth patterns and cytoplasmic streaming. Students can be involved by feeding the slime mould oat flakes.

SOP: Preparing a bacterial lawn culture

Aseptic technique is an important technique to use when preparing for and undertaking any microbiological procedure. In this activity, students use aseptic technique to lawn inoculate an agar plate

SOP: Streak plate inoculation

Inoculating a culture media with an inoculating loop to obtain isolated colonies.

SOP: Susceptibility testing of antiseptics and disinfectants

This activity uses aseptic technique and culture inoculation techniques to test the effectiveness of antiseptics and disinfectants on bacteria.

SOP: Gram stain a microbial culture smear

Gram stains are used to differentiate and identify different bacteria. This activity involves the preparation of a culture smear on a slide, the Gram stain and subsequent microscopic examination of the stained smear

Chapter 4 Safety in the school laboratory—protocols

Systems and protocols for safety during microbiological activities need to be planned and implemented prior to commencing laboratory activities. Teachers and laboratory technicians must enforce standard microbiological techniques including aseptic techniques, microbiology laboratory rules and spills procedures in order to maintain safety.

It is imperative that procedures for microbiological safety be followed closely. This includes the prevention of transmission of disease caused by poor microbiological technique. The most probable routes of transmission in school microbiology are:

- direct skin, eye or mucosal membrane exposure to an agent
- inhalation of infectious aerosols.[22]

Many cases of laboratory-acquired infections can be prevented by the use of good microbiological technique acquired through training programmes.

4.1 Training of staff

Teachers and laboratory technicians undertaking microbiological procedures should receive appropriate training prior to commencing this work unless they have prior knowledge, skills and experience. Staff need to demonstrate good microbiological technique, competency and confidence when performing procedures, interpretations and outcomes of activities, and competency in microbiological hazard awareness to maximise the student experience in microbiology. Staff who have completed microbiological training from higher educational facilities and/or relevant workplaces generally have the skills and knowledge sufficient for the school setting.

Good microbiological technique requires an understanding of aseptic procedures and the strict adherence to working methods designed to eliminate or minimise exposure to potentially infectious material via aerosols, splashes, ingestion, absorption and accidental inoculation.[23]

Training should be provided for staff who will then be able to teach students. The trainer should be a person skilled and experienced in microbiology. Alternatively, there are external training providers (and some universities) who can deliver this training.

Science ASSIST recommends the following training practices for consideration by Australian schools. The practices are adapted from *Guidelines for Biosafety in Teaching Laboratories* published by American Society for Microbiology (2012).

Comprehensive microbiology training should achieve the following outcomes:

- The trainer provides extensive initial training for teachers and laboratory technicians to:
    1. cover the safety hazards of working with microorganisms
    2. develop competency in microbiological practices and techniques required for work in Physical Containment 1 (PC1) laboratories using Risk Group 1 (RG1) microbes.
    3. develop skills and confidence with these practices and techniques to be able to demonstrate to students how to handle microorganisms safely and responsibly minimising hazards and risks.
- Provide refresher training annually and/or whenever a new procedural change is required.
- Ensure staff and students are aware of rules and regulations in their jurisdiction relating to the handling and cultivation of microorganisms.

- Ensure staff are competent in carrying out risk identification, assessment and management of controls, and to document these in a risk assessment.
- Emphasise to students the importance of reporting accidental spills and exposures.
- Keep a biosafety manual specific to the laboratory and/or unit of work in the laboratory.

Records of successfully completed staff training should be established and maintained within the school.

4.2 Microbiology laboratory rules

Working safely in microbiology necessitates that staff and students be aware of, and follow, microbiology laboratory rules and specific work practices. These are in addition to laboratory safety rules followed in general science laboratories. The following work procedures are the minimal requirements for a school microbiology laboratory. Each school should develop its own policy in regards to microbiology laboratory rules.

- Science ASSIST strongly recommends that all science rooms be locked unless a teacher or other authorised person is present.[24] Laboratory access by students and visitors should be under supervision only.
- Since microbiology activities are not conducted in every laboratory all year round, Science ASSIST suggests that a sign only be displayed at the entrance to the laboratory and/or preparation rooms when microbiological agents are being handled, particularly for school level 3 activities. The sign should be removed when biohazards are no longer present. This sign should include the biohazard symbol and laboratory containment level. Any access restrictions, plus contact information for responsible persons, should also be included.
- A biohazard symbol should also be displayed on equipment such as incubators, fridges, freezers or containers when microorganisms are being stored. Again, this can be removed when the biohazard material has been removed.

  The AS/NZS 2243.3:2010 *Safety in Laboratories – Microbiological safety and containment* states:

  '5.2 REQUIREMENTS FOR PC1 LABORATORIES

  5.2.1 General

  'A sign complying with Appendix D showing the level of containment, together with hazard symbols as appropriate and any access restrictions should be prominently displayed at the entrance.'

  The biological hazard symbol per Appendix D is a black symbol on a yellow background. General microbiology laboratories should also have the laboratory containment level indicated on the sign in black letters as below:

- Do not commence work unless a site-specific risk assessment is completed and control measures are implemented.
- Mouth pipetting is prohibited.
- Eating, drinking, and the application of cosmetics are prohibited.
- Storage of food and drink in laboratory refrigerators is prohibited.
- PPE will comply with AS/NZ 2243:1. Lab coats or disposable aprons, which protect the front of the body should be worn. Long hair must be tied back.
- Hands must be thoroughly washed before commencing activities and before leaving the laboratory.
- Gloves are worn if there is a cut or wound on the hand, and discarded after use with infectious laboratory waste. The cut or wound should also be covered with a band-aid or other dressing.
- All bench surfaces should be disinfected with 70% ethanol prior to and after handling microorganisms.[25]
- Precautions must be taken to ensure reading and writing materials do not become contaminated.[26] Keep the work area free of non-essential materials.[27]
- Good laboratory hygiene must be maintained and aseptic technique should be used when culturing microorganisms.
    - Do not breathe on cultures of agar plates. Keep conversation to a minimum.
    - Do not touch face or other parts of body.
    - Do not touch contaminated areas such as windows, doorknobs, seats, benches etc. with sterile equipment such as bacteriological loops.
- Cultures must be clearly identified, dated and appropriately stored, and should remain on open benches no longer than is necessary.
- Aerosols must be controlled to minimise their production especially on open benches. When using a loop, draw it from the cooler to the hottest parts of the Bunsen burner flame or use a hooded or electric Bunsen burner.
- Prior to incubation, Petri dishes should be sealed and incubated under aerobic conditions.
- Laboratory waste contaminated with microorganisms must be decontaminated prior to disposal in either an autoclave or appropriate pressure cooker.
- Work benches must be decontaminated immediately following spills.
- Communally used items and furniture such as doors, cupboards, telephones and keyboards must be regularly cleaned and disinfected.
- Staff and students must remove laboratory coats and thoroughly wash their hands before moving to areas outside the laboratory.

While conducting their experiments, students should never contaminate items that will leave the laboratory. The area for culturing and working with microorganisms should be as separate as possible from the area for taking notes or using laptops. Students should write with laboratory-use-only pens and pencils during a practical activity. Always minimise the number of notebooks and/or lab manuals on the lab bench. All other personal belongings should be stored away from the work area in spaces approved by the teacher. Papers that students will take home should be protected from contamination during the lab period. Optional approaches for taking notes in the lab are dependent upon the design of the facility, and practices will vary from school to school.

Instructions for practical activities should be laminated pages that are disinfected after each class and remain stored in the lab. A desk copy can be kept at each lab table for use during class. Alternatively, practical activity instructions can be displayed from the teacher's laptop via data projector for class use.

These microbiology laboratory rules and specific work practices form part of a staff training package and students should understand these rules before commencing any microbiological studies. These rules can be laminated and displayed in each microbiology laboratory. A printable version is in the Appendix 3 of this guideline.

4.3 Hand washing and hygiene

Hand washing is an integral part of establishing a clean microbiology working environment, and an important hygiene practice when working in the laboratory. Hand washing must be performed thoroughly prior to and after any microbiological activity.

AS2243.3: 2010 recommends the use of Chlorhexidine (0.5-40% w/v) in alcoholic formulations for 2 minutes for skin disinfection. In the context of a school laboratory handling RG1 microbes, commercially available anti-bacterial liquid hand wash is an option and is preferable to soap. Schools should consult the relevant safety data sheet when selecting a suitable hand wash product.

Liquid hand wash pump packs should be placed at sinks designated for hand washing. A hand-washing sink should be provided in each laboratory, preferably near the exit door. Elbow or foot operated tap handles should be considered when planning new or refurbishing laboratories.

Paper towel should be used in preference to reusable cloth towels. Paper towel dispensers should be located as close as practicable to the hand-washing sink. Used paper towel should be collected in a waste bag for disposal to an industrial waste bin. Hands should be dried thoroughly to ensure no contaminants remain in the cleansing liquid.

A printable version of hand wash technique published by the World Health Organisation is available at http://www.who.int/gpsc/5may/How_To_HandWash_Poster.pdf

4.4 Protective clothing and equipment

Personal protective equipment (PPE) refers to a variety of barriers, used alone or in combination, to protect mucous membranes, airways, skin and clothing from contact with infectious agents. PPE used as part of standard precautions includes aprons, gowns, gloves, surgical masks, protective eyewear and face shields.[28] A site specific risk assessment will highlight items of PPE that are required to minimise hazards in school microbiology.

- Personal protective equipment required for use in school PC1 labs using RG1 microbes:
  - Single-use plastic aprons that provide protection to the front part of the body shall be worn within the laboratory. Disposable aprons should be disposed of immediately after the microbiological activity in a contaminated waste bag to be double bagged and disposed in an industrial bin.
  - Closed leather footwear shall be worn.
  - Safety glasses or goggles shall be worn to protect eyes from splashes and other hazards.
- Gloves should only be worn if the person has any cuts or skin problems such as dermatitis. They could obstruct some activities and create other hazards if using a Bunsen burner. Gloves are not required for standard microbiological procedures if proper hand hygiene is performed. Proper hand hygiene involves thorough hand cleansing prior to and immediately after finishing handling microorganisms and any time that microbes accidentally contact the skin.[29]

Safety glasses are intended to provide eye protection against common laboratory hazards such as chemical and biological splashes and low impact energy activities. The lenses must be appropriate to the protection required. Note while it is possible to obtain prescription safety glasses, normal prescription glasses are not considered safety glasses as they do not provide protection against splashes.[30] Safety glasses should be cleaned regularly according to the manufacturer's instructions, generally with detergent solution, and be completely dry before being stored.

Staff and students should don PPE in the following order:

1. apron or lab coat
2. safety glasses
3. disposable gloves, if required

PPE must be removed before leaving the laboratory in the following order and stored within the laboratory.

1. disposable gloves, if used. (For 'Removal of gloves technique' see http://education.qld.gov.au/health/pdfs/healthsafety/handsgloves.pdf)
2. safety glasses
3. apron or lab coat

Perform hand hygiene immediately after removing PPE.

Chapter 5 A clean environment—decontamination and sterilisation

5.1 Importance of a clean work space

A good knowledge of how to control microorganisms (kill, inhibit or remove) in an environment is important to adequately manage microorganisms in the laboratory.

Standard practice in microbiology is to create a clean workspace prior to commencing microbiological activities. This involves the sterilisation and/or disinfection of equipment, instruments and work surfaces, the use of sterile growth media, and use of practical techniques that will minimise the chance of introducing microbial contaminants into sterile cultures or media.

By using certain physical agents, physical processes or chemical agents, microorganisms can be killed, inhibited or removed.

5.2 Decontamination and sterilisation

5.2.1 Decontamination

Decontamination can be defined as a process that renders an environmental area, device, item, or material safe to handle (i.e., safe in the context of being reasonably free from a risk of disease transmission) and to make (an object or area) safe for unprotected personnel by removing, neutralising, or destroying any harmful substance.[31]

Decontamination in the microbiology laboratory must be carried out with great care. Decontamination may entail sterilisation, disinfection or antisepsis.

Regardless of the method, the purpose of decontamination is to protect the laboratory worker, the environment, and anyone who enters the laboratory or handles laboratory products away from the laboratory.

In laboratory settings, all contaminated equipment or apparatus, spent laboratory materials, and regulated laboratory wastes should be decontaminated before being washed, stored or discarded. This is preferably accomplished by a sterilisation procedure known as steam autoclaving, perhaps the most cost-effective way of decontaminating a device or an item

5.2.2 Sterilisation

Sterilisation is to make free from bacteria or other living microorganisms.[32]

Any item, device, or solution is considered to be sterile when it is completely free of all living microorganisms including spore formers and viruses. A sterilisation procedure is one that kills all microorganisms, including highly resistant bacterial endospores. Sterilisation can be accomplished by dry heat (hot air ovens), steam under pressure (autoclaves or pressure cookers), incineration (flaming loops) and radiation (gamma sterilisation of plastic ware).[33] Sterilisation in the school laboratory is commonly achieved using an appropriate pressure cooker or autoclave to heat the items to a temperature of 121°C for 15–20 minutes at 15psi (pounds per square inch of pressure).

Microbiological items that are sterilised by steam sterilisation include:

- molten agar after preparation and prior to pouring into petri dishes
- heat resistant equipment items—forceps, hockey stick spreaders, glass petri dishes, glass bottles that the manufacturer deems suitable for sterilisation

- equipment that has been contaminated by exposure to microbes or during microbiological procedures and prior to washing—forceps, hockey stick spreaders, glass petri dishes, glass bottles that the manufacturer deems suitable for sterilisation
- inoculated or contaminated agar plates broths or slopes prior to disposal
- surplus agar plates broths or slopes
- surplus or redundant stock cultures
- other microbiological waste prior to disposal such as microbial samples, contaminated cotton swabs, contaminated paper towel and items used for spill clean up. Note you should not put anything soaked in chemicals such as 70% alcohol or sodium hypochlorite through an autoclave or pressure cooker.

Sterilisation of these items ideally takes place as soon as possible after completion of a practical activity. Place chemical sterility indicator strips in the centre of every load. Check the strip after the run is complete to ensure temperature and steam conditions have been met.

Items used in microbiology, may require sterilisation 2–3 times or more during the course of preparation for and performance of an activity, e.g. flaming loops.

Further information:

Efficient methods of decontamination and sterilisation in school laboratories are outlined in Science ASSIST:

- *SOP: Operating a pressure cooker and autoclave* (Appendix 1)
- *AIS: Decontaminate microbiological equipment* (Appendix 2)
- *AIS: Microwave, pressure cooker or Autoclave? Recommendations for best practice of sterilising agar* (Appendix 2)

5.3 Disinfectants/antiseptics

5.3.1 Disinfectants

Disinfectants eliminate nearly all recognized pathogenic microorganisms with the exception of bacterial spores on inanimate (non-living) objects.[34] Disinfection is less lethal than sterilisation[35] Use of a disinfectant does not necessarily result in sterility.[36]

In school laboratories, disinfection of benches and instruments should take place **before and after** microbiology procedures as part of aseptic technique. Good laboratory housekeeping dictates all benches should be cleaned regularly and thoroughly to maintain maximum hygiene and reduce the possible spread of infection or chemical contamination. 70% v/v ethanol is the preferred disinfectant for cleaning benches and surfaces not obviously contaminated.[37]

Microorganisms present a range of resistance to chemical disinfectants and no single disinfectant is effective in all situations.[38] Chemical disinfectants suitable for use in standard school experiments and procedures are restricted to substances that are safe for students and staff to use. This eliminates many disinfectants used in clinical or surgical environments. However, by using Risk Group 1 microbes, the risk of users contracting potentially harmful diseases is reduced or eliminated. Standard laboratory hygiene and safety procedures must be used with regard to dissections on animal tissues and use of microorganisms, and no human blood or body fluids should be used.

A site-specific risk assessment must be performed on the disinfection procedure, with consideration for the disinfectant to be used. A manufacturer's safety data sheet should be consulted in the formulation of risk assessments.

Bleach and hospital grade disinfectant can be purchased from a laboratory supplier or supermarket. Script on the front or main label of a container will indicate if a disinfectant is 'hospital grade'. Alcohol, (or its alternatives: methylated spirit, ethanol and isopropyl alcohol) is purchased from cleaning suppliers, hardware stores or laboratory suppliers. Special storage for flammable substances is required.

Check labels of disinfectants to determine the active component and its concentration.

All disinfectants should be prepared according to the manufacturer's instructions. Dilutions should be in fresh water using clean glassware and stored in clean and appropriately labelled storage containers. Fresh disinfectant should be prepared regularly.

5.3.2 Antiseptics

Antiseptics are chemical agents that slow or stop the growth of microorganisms on external surfaces of the body and help prevent infections. Antiseptics should be distinguished from antibiotics that destroy microorganisms inside the body, and from disinfectants, which destroy microorganisms found on inanimate (non-living) objects. Some chemical agents can be used as both an antiseptic and a disinfectant. The purpose for which it is used is determined by its concentration.[39]

In the context of school microbiology, an antiseptic may be used in the decontamination and treatment of human skin after exposure to a microbial culture.

5.3.3 Disinfectants and antiseptics recommended for use in schools

There are multi-purpose chemical agents that are suitable disinfectants and antiseptics for most routine procedures performed in a school science laboratory [Physical Containment 1 (PC1) laboratories, using Risk Group 1 (RG1) bacteria (non-sporing)].

Table 2: Disinfectants and antiseptics suitable for use in Physical Containment 1 (PC1) laboratories, using Risk Group 1 (RG1) bacteria (non-sporing).

Type of disinfectant	Suitability	Limitations	Recommended instructions
70% (v/v) ethanol or isopropyl alcohol	Dissections Microbiology Smooth hard surfaces (i.e. laboratory benches) Gram negative microorganisms Skin Disinfectant	Flammable volatile, high cost. May harden rubber products. Inactive against spores	Dilute to 70% in fresh water. Must be in contact with material being disinfected for at least 10 minutes, longer for heavily soiled items. Industrial methylated spirit (95% ethanol) or isopropyl alcohol appropriately diluted is an acceptable alternative.
Sodium hypochlorite (household liquid bleach)	Disinfecting reusable microbiology equipment after use and single use equipment prior to disposal. Effective against a	Poor stability – prepare immediately prior to use. Affected by temperature, concentration and pH. Bleach solutions are	Contaminated (but not visibly soiled) work surfaces treat with a 0.5–1% v/v (5000–10000 ppm) (available chlorine) solution for at least 10 minutes. General work surfaces

	wide variety of microorganisms	corrosive to metal objects and may damage rubber items. Do not autoclave any materials soaked in bleach solution, as there is a risk a toxic gas may be produced. Bleach is an irritant to users at higher concentrations (skin and mucous membranes). Activity of chlorine bleach is reduced by organic matter[40] Increased concentrations may be required. May bleach and damage clothing. Less effective against spores. Low cost.	may also be routinely cleaned with 0.5–1% v/v (5000–10000 ppm) (available chlorine). Soak microbiology equipment and disposables in 0.5–1% v/v (5000–10000 ppm) (available chlorine) for at least 10 minutes, longer for heavily soiled items. Do not use on metal or rubber items SPILLS: wads of absorbent paper towel soaked in 0.5–1% v/v (available chlorine) hypochlorite solution may be used to absorb and/or wipe down the area affected by a spill. For infectious spills see procedure Section 5.6.2.
Hospital grade disinfectant	Cleaning smooth hard surfaces. Use after general lab procedures, bench and lab cleaning, dissections, spills, cleaning large areas.	Not effective on spore formers.	Dilute according to the manufacturer's instructions in fresh water. Use in a spray or wash bottle to clean benches.
Chlorhexidine	Gram positive, gram negative bacteria and fungi.[41] Skin disinfectant.		0.5–4% (v/v) in ethanol. Wash hands in solution for 2 minutes

5.4 Preparation of sterile and clean resources for microbiological activities in schools

(NB: This section is duplicated in the *AIS: Preparing sterile equipment for microbiology* that is available as a stand-alone document in Appendix 2)

Equipment used in microbiology should be sterile before using. This enables aseptic techniques to be used when transferring microorganisms for inoculation, sampling environmental areas, adding susceptibility discs to agar plates and Gram staining.

This equipment should be prepared before the class activity and stored in clean, lidded containers.

Equipment such as hockey stick spreaders, inoculating loops and sterile swab sticks can be purchased as single-use items from commercial scientific suppliers if the school budget allows or it is more time effective to do so.

Good organisational skills and a disciplined approach ensure that every activity is performed both safely and successfully.[42]

Sterile equipment is prepared well in advance to the planned activity day. Staff preparing items for microbiological activities should carefully study which items such as forceps, hockey stick spreaders and quantities needed before sterilisation can take place. Many schools prepare sterile items and store them in sterile lidded containers until required. Sterile equipment can be stored indefinitely if the packaging is not breached. Sterile agar can be stored indefinitely if it remains in the container it was sterilised in, and the container has not been reopened. Agar plates are best prepared 1–2 days before an activity, and not stored for more than 4–6 weeks. Most schools do not have access to facilities that enable agar plates to be poured in total aseptic conditions. Agar plates purchased commercially may be stored longer if contamination has not occurred.

In-house preparation of sterile items is cost effective to schools as some pieces of equipment can be repeatedly recycled. Care should be taken with ethanol, as it is a flammable substance and should not be used near a naked flame.

Considerations:

- Sterilisation of equipment should be performed in a draught-free area.
- Items to be sterilised should be clean and dry, metal forceps should not be rusty, glass items should not have chips or cracks.
- Consult the planned activity or activities prior to sterilising items to ensure there is the required number of items available during the activity.
- Soaking items in a container of 70% (v/v) ethanol for 10 minutes, disinfects/decontaminates, but does not sterilise items.
- Ensure the bench area for this purpose has been decontaminated with 70% ethanol prior to commencing.
- Aluminium foil or greaseproof paper may be used to wrap items to be sterilised.
- Sterile items can be stored in a large lidded plastic container that has been decontaminated with ethanol and paper towel.
- **Glassware and metal instruments can be wrapped in aluminium foil and sterilised using dry heat in an oven at 160°C for 2–3 hours.[43]**
- **All sterilising processes using an autoclave/steriliser or pressure cooker should be at 121°C for 15–20 minutes at 15psi.**
- Professional microbiologists and higher education providers promote the sterilisation technique of ‘flaming’ hockey stick spreaders and forceps prior to using by dipping in 70% ethanol and igniting it in the Bunsen flame. Incorrect techniques can encourage microbial aerosol transmission and risk the ethanol catching on fire. **Science ASSIST does not recommend this practice in the school setting, but instead recommends sterilising these items in an autoclave or an oven.**

Table 3: Suggested and alternative sterilising techniques		
Item	**Suggested sterilising technique**	**Alternative technique**
Sterile plastic Petri dishes	Purchase sterile, leave wrapped in original packaging until required. (Do not autoclave prior to use. Plates do not retain shape when autoclaved.)	
Sterile glass Petri dishes	Wrap glass Petri dishes in greaseproof paper or aluminium foil and sterilise in an autoclave	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2–3 hours
Nutrient agar plates	Prepare agar solution according to the manufacturer's instructions,!autoclave in a heat-safe bottle with lids loose and pour plates when temperature of sterile agar is ~50^0C using aseptic technique. When set, wrap in plastic wrap. Store at 4°C until required.!See ASSIST SOP: Preparing agar plates	Purchase prepared and sterile from a biological supplier
Nutrient broth	Prepare broth solution according to the manufacturer's instructions. Aliquot ~15mL into McCartney bottles (28mL capacity) keep lids loose. Autoclave. When cool tighten lids and store at 4°C until required.	Purchase prepared and sterile from a biological supplier
Sterile water	Aliquot 2mL into Bijou bottles (7mL capacity) keep lids loose. Autoclave. When cool tighten lids and store at 4°C until required.	
Sterile plastic dropping pipettes	Purchase single-use pipettes from commercial scientific, biological or medical suppliers.	
Sterile swab stick	Purchase sterile, leave wrapped in original packaging until required.	Autoclave cotton buds in foil covered beaker.
Sterile 'L' spreader	Wrap in aluminium foil and autoclave. Store until required.	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2-3 hours.
Sterile forceps	Wrap in aluminium foil or place inside a clean test tube, cover opening with aluminium foil and autoclave. Store until required.	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2-3 hours.
Sterile test tubes/ conical flasks	Cover opening with foil or plug with non-absorbent cotton wool. Autoclave.	Cover opening with aluminium foil. Sterilise using dry heat in an oven at 160°C for 2–3 hours
Inoculating loop	Flame to red heat in the blue flame of the Bunsen burner.	Purchase sterile disposable inoculating loops, leave wrapped in original packaging until required.

Further information: See *SOP: Operating a pressure cooker and autoclave* (Appendix 1).

5.5 Decontamination of equipment and cultures at the conclusion of microbiological activities

Immediately concluding microbiological practical activities, it is imperative that thorough decontamination of equipment, the laboratory, and the sterilisation of bacterial cultures and agar plates occur. This prevents contamination of future microbiological and non-microbiological practical activities and possible ill effects of students and staff.

Staff and students each have an important role.

Staff responsibility:

- Provide containers, and contaminated waste bags e.g. autoclave bags or oven bags for students to segregate and dispose of equipment and cultures. Each receptacle should be labelled with its function. Ensure these are removed from the laboratory at the end of the activity.
    - Prepare 0.5–1% v/v (5000–10000 ppm) (available chlorine) sodium hypochlorite (bleach) solution in a large (500–1000mL) labelled beaker (1 per student group) for swab sticks. Provide one for each student group.
    - Dilute hospital-grade disinfectant according to the manufacturer's instructions for contaminated items such as forceps. Place diluted disinfectant in a large labelled beaker and provide one for each student group.
    - Provide a solid bin containing autoclave bags or oven bags.
    - Provide a separate autoclave bag for contaminated disposable PPE and reusable PPE.
- Ensure all agar plates and equipment are returned to the designated area.
- Oversee thorough bench decontamination and hand washing of students.

Student responsibility:

- Return all agar plates after examination. Place directly into designated autoclave or oven bags.
- Place all equipment in the designated area.
- Clean benches by swabbing with hospital grade disinfectant or 70% ethanol using disposable paper towel. Allow to air dry.
- Dispose of aprons, gloves and bench cleaning paper into the appropriate contaminated waste autoclave bag.
- If lab coats, aprons or uniforms are contaminated, they will need to be bagged and sterilised before washing and reuse.
- Wash hands before leaving the laboratory or beginning a new activity.

Contaminated waste includes any item that has been exposed to microorganisms in the course of a practical activity. Non-contaminated waste is any item that has not been exposed to any microbes such as freshly prepared un-inoculated agar. If in doubt, non-contaminated waste should be treated as a contaminated item.

5.5.1 Disposal of microbiological waste

All microbiological waste is required to be sterilised before disposal. Microbiological waste consists of inoculated agar plates, broths, used swabs or other items used to sample or manipulate microorganisms. Plastic Petri dishes are commonly made from clear polystyrene plastic which are heat resistant up to 80^{o}C.[44] They are regarded as a use once disposable item and under sterilisation conditions in an autoclave or pressure cooker they will deform.

The best way to sterilise non-liquid biological waste in an autoclave or pressure cooker is by placing them into a bag that will withstand the sterilisation conditions and contain the treated contents. Schools have the option of using two different types of bags:

- Autoclavable biohazard bags, which are available from scientific suppliers, see the Science ASSIST *School science suppliers* list. These are made from a heavy-duty plastic e.g. polypropylene marked with the international biohazard symbol and usually have the word autoclavable written on them. They are available in a variety of sizes.
- Oven bags which can be purchased from supermarkets. These are also available in different sizes.

If you are unsure if your biohazard bags are suitable for autoclaving, we suggest that you contact the supplier for advice.

Procedure for using an autoclavable biohazard or oven bag for sterilising microbiological waste:

- **Loosely pack microbiological waste including agar plates into bags to no more than 2/3 full**. This will ensure that the steam during sterilisation will penetrate the entire load. Bags that are tightly filled to capacity will not allow effective steam penetration and the contents will not be sterilised even if all sterilisation parameters are met.
- **Make sure there are no sharp objects present** that may puncture the bag.
- **Loosely tape shut the bag leaving an opening of about 5–6cm** to allow good steam penetration. This can be done with autoclave tape or a rubber band. Never tightly close the bags as they are impervious to steam and therefore the temperature of the inside of the bag will not be sufficient for sterilisation.
- It is advisable to **place the bag into a secondary container** within the steriliser to prevent any leakage into the steriliser should the bag rupture. The container must be able to withstand the autoclaving conditions.
- **Do not overload the steriliser** with too many bags as this may block steam circulation.
- **Use a sterility compliance strip** to indicate if the correct time, temperature and pressure have been reached during the run time. These are available from scientific suppliers.
- **Sterilise at 15psi, 121^{o}C for 15–20 minutes.**
- After sterilisation has been verified, the autoclave or oven bag containing waste items should be **disposed of by placing it into a sturdy garbage bag which is sealed for immediate disposal in industrial bins.**
- **Wear heat protective gloves** when removing waste from the steriliser.
- **Sterilisation of these items ideally takes place as soon as possible** after completion of a practical activity and occurs within the science laboratory or prep room area.

Liquid cultures in bottles or test tubes can be placed onto a tray in the steriliser with their lids loose. See Science ASSIST *SOP: Operating a pressure cooker and autoclave* in Appendix 1.

5.5.2 Decontaminating equipment and facilities

There are a variety of methods used to decontaminate equipment and facilities used in microbiological activities.

Table 4: Decontamination procedures for equipment and facilities used in school microbiology laboratories.	
Contaminated item	**Suggested decontamination technique**
Inoculated agar plates – plastic	Pack unopened plates loosely in autoclave bag, leaving an opening of about 5–6cm to allow good steam penetration. Autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.
Inoculated culture broth in McCartney or Bijou bottles.	Make sure that the lids are loose. Autoclave at 121°C, 15psi for 15–20mins. Empty contents in the sink with copious amounts of water. Wash in warm soapy water, rinse well and dry. *Resterilise:* Re-autoclave loosely lidded container. Store in a clean closed container.
Inoculated water in glass bottle	Make sure that the lids are loose. Autoclave at 121°C, 15psi for 15–20mins. Empty contents in sink. Wash in warm water and dry. *Resterilise:* Re-autoclave loosely lidded container. Store in a clean closed container.
Plastic dropping pipettes	1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in the general waste.
Used swabs	1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours or. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15-20 mins. When cool, place unopened autoclave bag in a garbage bag and dispose in the general waste.
Sterile 'L' spreader	1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours, or 2) Place directly into an autoclave resistant container and cover with foil or place into an autoclave/oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15-20 mins. 3) After sterilisation, wash in warm soapy water, rinse and dry. *Resterilise:* Wrap in foil and sterilise in an autoclave or hot air oven. Store until required for re-use.
Sterile forceps	Carefully place into an autoclave resistant container such as a large test tube, cover with foil and autoclave. Wash in warm soapy water, rinse and dry. *Resterilise:* Wrap in foil or place inside a clean test tube, cover opening with foil and autoclave, Store until required for re-use.
Test tubes	Autoclave at 121°C, 15psi for 15–20mins. Empty contents in sink. Wash in warm soapy water, rinse and dry. *Resterilise:* Plug with non-absorbent cotton wool and autoclave or cover the opening of the test tubes with foil and sterilise in a hot air oven or autoclave. Store in a clean closed container.

Contaminated item	Suggested decontamination technique
Inoculating loop	Flame to red heat carefully in the blue flame of the Bunsen burner to prevent the transmission of aerosols. Cool and reuse immediately. Alternatively, if using disposable inoculating loops, 1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours.. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.
Susceptibility discs Mastrings (set of 6 or more antibiotic discs joined together)	Susceptibility discs and Mastrings should remain on the agar plate after examination. The agar plate remains closed. Pack unopened plates loosely in autoclave bag and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.
Paper towel exposed to contaminated areas Used disposable aprons/lab coats Used gloves	If not soaked in bleach or alcohol, sterilise in an autoclave or pressure cooker. If soaked leave for the recommended time and then dispose of into the general waste. An autoclave or oven bag should be placed in the laboratory for students to place these waste items directly into the bag. Do not overfill the bag. Leave an opening of about 5–6cm to allow good steam penetration and sterilise for 15–20 min at 121°C and 15psi. Place the unopened autoclave bag into a sturdy garbage bag and seal for immediate disposal in an industrial bin.
Laboratory benches Plastic containers used for storage and distribution of equipment Any other hard surface	Dilute disinfectant in fresh water according to the manufacturer's instructions. Use in a spray bottle. Dilute ethanol to 70% in fresh water, use in a wash bottle. Ethanol is flammable. Apply liberally to laboratory bench or other hard surface to be decontaminated. Wipe lightly with paper towel. Allow the residual to air dry.

5.6 Spill kits

Spill kits should be present in all work places that store and use hazardous materials. In the school microbiology laboratory, this includes hazards such as chemicals and biohazards. The type of spill kit used should reflect the type of hazards present in the laboratory, e.g. a chemical spill kit for a laboratory storing and using chemicals, and/or a biohazard spill kit for a laboratory handling microbiological, human or animal fluids.[45] Spill kits in school microbiology laboratories need to specifically include clean-up substances for:

- microbiology biohazards,
- flammable liquids, and
- chemical cleaning agents.

5.6.1 Biohazard spill kit contents

A microbiology specific spill kit for biohazard materials should include the following items:

Table 5: Items in a microbiology specific spill kit

	Item	Description
PPE	Eye protection	Wrap around type that fit over prescription glasses
	Nitrile gloves	Chemical resistant
	Apron or lab coat	Disposable options available
	Face mask	Useful protection when sweeping dusts/powders and when handling neutralising agent powders. Protection against aerosols.
Other items	Dustpan and brush	For sweeping up spilt powders
	Tongs/forceps	To pick up contaminated materials, e.g. fragments of broken glass containers. Sharps container available for use.
	Absorbent materials (i.e. paper towels)	To soak up small chemical or biohazard spills
	Autoclave or oven bags	To contain absorbed spills and biological waste.
	Signage	For example, “Do not enter: Biohazard spill clean-up in progress”
	Sodium hypochlorite (bleach) solution freshly prepared.	Absorb the treated spill with paper towel and flood with 0.5–1% sodium hypochlorite to sterilise prior to disposal. Also, use bleach to treat exposed surfaces.
Absorbent materials, pads and pillows for chemical spills. Select as appropriate from the following range of options.	Vermiculite	Silicate material that can absorb up to 10 times its weight of water. Good for aqueous spills, less effective for oil based spills.
	Attapulgite (‘kitty litter’)	Silicate (clay) mineral that is an excellent absorption agent. Useful for oil-based spills
	Commercial chemical absorbent pads and pillows	Available in various sizes and shapes-pads, pillows, snakes
	Super-absorbent polymers	Polymers with cross-linking to form gels that can absorb many times their weight of aqueous solutions. The material in disposable diapers
	Sand	Useful to contain a spill

Schools must ensure the availability of current Safety Data Sheets (SDS) prepared by the manufacturer or importer for all hazardous chemicals.

5.6.2 Spill clean-up procedure for biohazards

For minor spills:

1. Wear gloves and protective clothing.
2. Cover the spill with paper towels to contain and absorb the spill.
3. Pour an appropriate disinfectant over the paper towels and the immediate surrounding area (generally, freshly prepared 0.5–1% v/v bleach solution is used). Leave for 30 minutes.
4. After the appropriate amount of contact time the soaked paper towel can be placed into the normal bin.
5. Wash the decontaminated area with water and detergent.
6. All contaminated material should be placed into either an autoclave bag or oven bag to be put through an autoclave or pressure cooker. This may include a lab coat or apron or uniform, if contaminated.
7. Do not autoclave material containing hypochlorite, since chlorine gas can be produced.
8. Wash hands thoroughly with soap and water on completion.

In the event of a large spill of infectious or potentially infectious material, the following spill clean-up procedure should be used.

1. Evacuate and isolate the immediate area, minimise any draughts.
2. Wear gloves and protective clothing, including face or eye protection.
3. Check for sharps from broken glassware. If present, remove with forceps and place into a sharps biohazard waste container. Wrap forceps in foil or place in a clean test tube, cover opening with foil and autoclave.
4. Cover the spill with paper towels to contain and absorb the spill.
5. Pour an appropriate disinfectant over the paper towels and the immediate surrounding area (generally, freshly prepared 0.5–1% v/v bleach solution is used)
6. Apply disinfectant concentrically beginning at the outer margin of the spill area, working toward the centre. Leave for 30 minutes.
7. After the appropriate amount of contact time, the soaked paper towel can be placed into the normal bin.
8. Wash the decontaminated area with water and detergent.
9. All contaminated material should be placed into either an autoclave bag or oven bag to be put through an autoclave or pressure cooker. This may include a lab coat or apron or uniform if contaminated.
10. Do not autoclave material containing hypochlorite, since chlorine gas can be produced.
11. Ensure that all materials are disposed of correctly and seek further advice if necessary.
12. Wash hands thoroughly with soap and water on completion.
13. Report the spill to the head teacher and/or the school Work Health and Safety Officer.
14. Staff and students should not be allowed into the area of the spillage, nor should the notice be removed until all the spilt substance is cleaned up and odours dissipated.

5.6.3 Spill clean-up procedure for chemicals

Major spills should be immediately referred to trained emergency personnel. Remove ignition sources and evacuate the building. It is helpful if the substance has been identified.

Minor spills, depending on the substances, may be treated as follows:

1. Notify laboratory personnel and neighbours of the accident.

2. Evacuate and isolate the immediate area.
3. If flammable liquid has been spilt, remove ignition sources and isolate power to the laboratory with the emergency switch.
4. Establish ventilation – open windows and doors.
5. If possible, positively identify the substance spilt. Check the labelling on the bottle identifying the chemical and its hazards. Look for hazard signal words and hazard statements and if possible, consult the safety data sheet.
6. Locate the spill kit.
7. Choose appropriate PPE (goggles, face shield, chemical resistant gloves, lab coat, apron).
8. Contain the spill using a spill absorbent material such as Vermiculite or sand as a barrier. Note: There are also commercial products available such as chemical absorbent pillows, either in pillow or long snake shapes, which can be used to contain spills.
9. **Acid and base spills** should be neutralized prior to clean up. Acids should be covered with sodium carbonate or sodium bicarbonate until the reaction ceases to fizz. Spilt bases should be neutralised with vinegar, boric acid or sodium bisulphite. Liberally sprinkle the neutraliser over the spill starting at the perimeter and continue towards the centre. Leave for 1–5 minutes.
10. **Flammable spills** should be covered with commercial spill absorbent material, chemical absorbent pads or paper towel to soak up the spill. These items should be then left to completely evaporate in a fume cupboard or a secure area outdoors away from all sources of ignition. Wash chemical absorbent pad or paper towel in water before disposal.
11. Mercury spills: in the event of your school having mercury, including mercury thermometers, it is important to have a prepared strategy for dealing with a potential spill. There are two approaches to this: Commercial Hg spill kits have absorbent sponges that pick up the globules and then stores the Hg in the collecting container when the sponge lid is screwed back on. There is also Hg decontaminant powder, supplied in the lists of chemicals, that reacts with the mercury to prevent the formation of vapour, which can then be collected for disposal.
12. Sweep solid material into a plastic dustpan and place in a sealed container.
13. Wet mop spill area. Be sure to decontaminate broom, dustpan.
14. Put all contaminated items (gloves, clothing, etc.) into a sealed container or plastic bag.
15. Ensure that all materials are disposed of correctly and seek further advice if necessary. Disposal of toxic substances may need to be arranged through a commercial waste disposal company
16. Return spill kit to storage location and arrange for used contents to be replaced.
17. Report the spill to the head teacher and/or the school Work Health and Safety Officer.[46]

Staff and students should not be allowed into the area of the spillage, nor should the notice be removed until all the spilt substance is cleaned up and odours dissipated.

After an incident, immediately replenish stock of spill kit, review procedures and effectiveness of the emergency plan, and document for WH&S reporting as per your school procedure. The references listed below have some very good guidance material.

For ‘Removal of gloves technique’ see http://education.qld.gov.au/health/pdfs/healthsafety/handsgloves.pdf)

For some school-based resources see:

- Victorian Department of Education and Early Childhood Development (DEECD). *Guidance Sheet 4: Chemical Spill Management,* DEECD website, https://www.eduweb.vic.gov.au/edulibrary/public/ohs/Guidance_Sheet_4_-_Chemical_Spill_Management.pdf (Accessed November 2016)

- ‘Spill Kits’, Western Australia Department of Education. Regional Technicians Group website, http://www.rtg.wa.edu.au/Spill%20kits/spills%20index.htm (Accessed November 2016)

Chapter 6 Media for cultivating microorganisms

Microorganisms grown in the laboratory require a culture medium that contains all the nutritional requirements for reproduction and growth. The main elements for cell growth include water, nitrogen source, carbon source, energy source, sulfur and phosphorus, certain inorganic ions and other growth factors such as vitamins.

6.1 What is culture media?

A culture medium provides the food and imitates the normal habitat for microorganisms and must contain carbon and energy sources and other nutrients. Different microorganisms use these elements in distinct ways to survive and thrive.

Microorganisms are diversified in their nutritional requirements. One type of culture medium will not support the growth of all microorganisms. Different species of microorganisms require different culture media to successfully grow and reproduce. Variations to the type and amount of specific nutrients result in different agar types and the microorganisms that grow on them.

Culture media can be solid (agar set in a petri dish) or liquid (broth). Many microorganisms will grow in either media. For observation purposes in school laboratories, solid culture media such as nutrient agar set in a Petri dish gives the best visual representation of the morphology, reproduction and growth pattern of different microbes without reopening the Petri dish for further examination.

6.2 Suitable media for schools

When selecting microbiological practical activities in schools, teachers and laboratory technicians should consider environmental, risk and staff competency factors.

Australian schools are generally designed and constructed to Physical Containment 1 (PC1) standard. Microbiological practical activities selected should align with facilities and work practices in a PC1 laboratory and use microorganisms only from Risk Group 1 (RG1). The use of culture media that are used specifically to grow microbes from RG2 or higher, must not be used in schools, as they can select for human pathogens (see section 6.4)

Nutrient and plain agar is recommended for most microbiological practical activities suitable for schools. Nutrient agar is a general-purpose, nutrient medium used for the isolation of bacteria and fungi. These agars do not selectively grow pathogenic bacteria and allow safe Risk Group 1 microorganisms to grow. They can also be used to demonstrate sensitivity of microbes to antibiotics and disinfectants.

6.2.1 Nutrient agar

Nutrient agar consists of peptone, beef extract, and agar, which is a complex carbohydrate derived from the extract of seaweed. Agar is an excellent solidifying agent because it liquefies at 100°C and solidifies at 40°C and is indigestible by microbes. Nutrient agar provides amino acids, minerals, and other nutrients used by a wide variety of bacteria for growth.

Ingredients of nutrient agar:

- 0.5% Peptone. Peptone is the principal source of organic nitrogen for the growing of bacteria.
- 0.3% beef extract/yeast extract. This contains water-soluble substances, which aid in bacterial growth, such as vitamins, carbohydrates, organic nitrogen compounds and salts.

- 1.5% agar. The solidifying agent. Agar is firmer and stronger than gelatine. It is still possible, however, to use gelatine as a culture medium for bacteria if agar is unavailable.
- 0.5% Sodium chloride. The presence of sodium chloride in nutrient agar maintains a salt concentration in the medium that is similar to the cytoplasm of the microorganisms.
- Distilled water. Water is essential for the growth of and reproduction of microorganisms and also provides the medium through which various nutrients can be transported.
- pH is adjusted to neutral (7.4) at 25°C.[47]

Nutrient agar can be made from dehydrated preparations available from scientific suppliers, using individual ingredients, or by purchasing pre-poured agar plates.

6.2.2 Plain or basic agar

Plain or basic agar is a non-nutrient agar used to culture the slime mould *Physarum polycephalum*. It contains a mixture of 1.5% agar and water. Alternatively, pre-poured plain agar plates can be purchased from biological suppliers.

Other culture media used for Level 1 microbiology activities may include:

- Algal medium – for cultivating algae
- Malt extract agar – for fungi and yeasts
- Paramecium culture medium
- Pond water solutions

These contain specific nutrients for the microorganisms and are suitable for use in schools.

6.3 Making plain/nutrient agar plates

Careful planning for microbiological activities includes preparing enough agar plates for the activity. Laboratory technicians preparing plain and nutrient agar plates generally should allow 20 minutes to make media, 45 minutes to autoclave and 60 minutes to cool before pouring plates. Setting time of the agar can range from 5 to 15 minutes.

Prior to pouring agar plates, keep sterile Petri dishes closed until agar is ready to pour into them. Air-born contaminants can easily invade an open Petri dish.[48] It is preferable to use sterile disposable plastic Petri dishes over glass.

Agar plates should be made up as required, and not stored for long periods to avoid any unwanted microbial growth. Agar plates should be disposed of as soon as possible after the activity.[49] All agars for microbiological work need to be sterilised before and after use. Alternatively, pre-poured sterile agar plates can be purchased from reputable biological suppliers.

Prepared agar plates should be stored in a refrigerator (below 4°C). Storage at temperatures higher than 4°C will reduce the shelf life. Do not freeze the plates, as this will denature the nutrient agar medium. Store upside down with the agar on the 'roof' of the plate. This prevents condensation forming on the agar surface and reduces the chance for contamination.[50]

For further information on the preparation of nutrient and plain agar see Science ASSIST *SOP: Preparing agar plates*. (Appendix 1)

For best practice for sterilising agar see Science ASSIST *AIS: Microwave, pressure cooker or autoclave? Recommendations for best practice of sterilising agar*. (Appendix 2)

Also, see Science ASSIST *SOP: Operating a pressure cooker and autoclave*. (Appendix 1)

6.4 Unsuitable media for schools

Agars used in health and industrial laboratories are used to grow specialised microbes under specific conditions. These are not suitable for the school laboratory. Selective, differential and enriched media such as blood, chocolate and MacConkey agars encourage the growth of more fastidious microorganisms, many of which are classed as RG2 and are pathogenic. **These media must not be used in school laboratories**.

Chapter 7 Inoculating agar plates

Inoculating media is a method where live microbes are transferred onto an agar plate. This is an area where contamination of the agar plate can occur. It is important to use aseptic techniques and follow the correct microbiological procedures when inoculating to prevent contamination.

A pure culture is a microbiological culture containing a single species of organism. In schools, this organism should be previously identified. An environmental (sometimes referred to as 'wild') culture contains a microbe that is grown from an unknown source such as those found in environmental sampling. The resulting microbe has not been identified in the laboratory. Remember always regard all microorganisms as potential pathogens, and use aseptic techniques at all times.

Where microorganisms are grown on agar plates from environmental sampling, the plates must not be opened and the microorganisms must not be subcultured. When observations are complete the plates should be sterilised in an autoclave or pressure cooker and then disposed of in the rubbish.

7.1 Inoculation techniques commonly used in schools

Microorganisms can be inoculated onto agar plates in school science laboratories by various methods.

7.1.1 Environmental sampling methods

- **Inoculation of plates via air exposure (settle plates)**
  Sterile Petri dishes are left open to the air in various places in the laboratory for a period of time before lids are replaced, sealed and incubated.
- **Inoculation by direct contact**
  Microorganisms are transferred directly to an agar plate by touching the surface of the agar with an item such as a coin. Touching the agar with your fingertips is not recommended as this could allow for the growth of pathogens.
- **Inoculation of plates with sterile swabs from environmental samples**
  Sterile cotton swabs are used to transfer microbes from an environmental area to an agar plate. Individually packaged sterile swabs may be purchased from a biological or medical supplier, or may be sterilised in an autoclave or pressure cooker prior to use. Place cotton buds in a beaker and seal with alfoil before sterilising.
  Sterile cotton swabs should be kept in the individually sealed packaging until required, and should never be unwrapped and placed on the laboratory bench unless the sampling area is the bench.
  A sterile swab is moistened in sterile water and wiped over the area to be sampled. The swab is then moved over the surface of the agar plate in a zigzag manner to transfer any microorganisms. The swab can be disposed of into the rubbish, as it has not been used to swab areas that contain any pathogens. See Science ASSIST *SOP: Microbes are everywhere* (Appendix 1).

Sample results can be seen in Chapter 9 Observing microbes

7.1.2 Methods using aseptic technique

- **Inoculation of plates with bacteriological loops**
  A heat sterilised loop or disposable inoculating loop can be used to sample from a yoghurt or cheese specimen or a liquid culture containing a RG1 microbe in nutrient broth. The!

inoculating loop is used to smear the sample either in a zigzag method over the surface of the agar as described above or placed onto a section of the surface of an agar plate and then streaked out for single colonies. See Science ASSIST *SOP: Streak plate inoculation* (Appendix 1).

- **Inoculation of plates to produce a Lawn culture**
  A liquid culture containing a RG1 microbe in nutrient broth is transferred to a sterile nutrient agar plate. One to two drops of culture are placed on the plate using a sterile dropping pipette, which is then immediately placed in a bleach solution. A sterile L-shaped spreader (Hockey stick) is used to gently spread the culture evenly over the agar creating a consistent 'lawn' of culture. The L-spreader is immediately placed into the bleach solution. See Science ASSIST *SOP: Preparation of a lawn culture* (Appendix 1).

Sample results can be seen in Chapter 9 Observing microbes.

7.2 Environmental sampling

When sampling from the environment, the microorganisms collected are not identified. It is therefore important to sample from environments that are not likely to contain human pathogens.

Suitable environments to obtain samples

- Laboratory bench
- Other fixtures within the laboratory e.g. window sills
- Coins from your pocket
- Pens or pencils
- Door handles
- Computer keyboards

Unsuitable environments to obtain samples

Areas where it is not recommended that samples be obtained as they may contain human pathogens.

- Toilets and toilet areas including floors, hand basins and taps
- Food preparation areas
- Any human or animal body fluids or carcasses
- Skin areas including the finger tips and the mouth
- Animal sources
- Soil samples

7.3 Aseptic technique

Aseptic technique is used in higher level activities to prevent contamination of pure cultures from foreign bacteria inherent in the environment. For example, airborne microorganisms (including fungi), microbes picked up from the lab bench-top or other surfaces, microbes found in dust, as well as microbes found on unsterilised glassware and equipment, etc. may potentially contaminate pure cultures, thus interfering with the lab results. Using proper aseptic technique can greatly minimise or even eliminate the risk of contamination.

Aseptic technique is used to:

- transfer cultures from one medium by inoculating another medium. This is called subculturing.
- prevent contamination of cultures and media from microbes in the environment

- prevent aerosols and drops of microorganisms from being spread in the environment and/or infecting staff and students.

7.3.1 Aseptic practices

- Wash hands with soap and water before and after working with microorganisms.
- Cover any cuts with a waterproof dressing and consider wearing disposable gloves.
- Make sure work surfaces are decontaminated before and after working with microorganisms with 70% v/v ethanol.
- Make sure inoculating instruments (inoculating loops and swabs) are sterilised prior and after use.
- Make sure that inoculating instruments containing microbiological samples are not allowed to touch any surface other than the agar that requires inoculation.
- Flame the mouth of all test tubes or bottles containing sterile water or microbial culture broth both when the cap is removed and before it is replaced.
- Plates should be open for a minimum amount of time to minimise the risk of introducing any contaminants from the air.
- Inoculation should be carried out as quickly as possible to minimise introducing any contaminants.
- Work close to the Bunsen flame as it provides an updraught that carries air away from the workspace, so reducing contamination from the air.
- Have a bacterial spills kit available.

Chapter 8 Incubation and growth of microorganisms

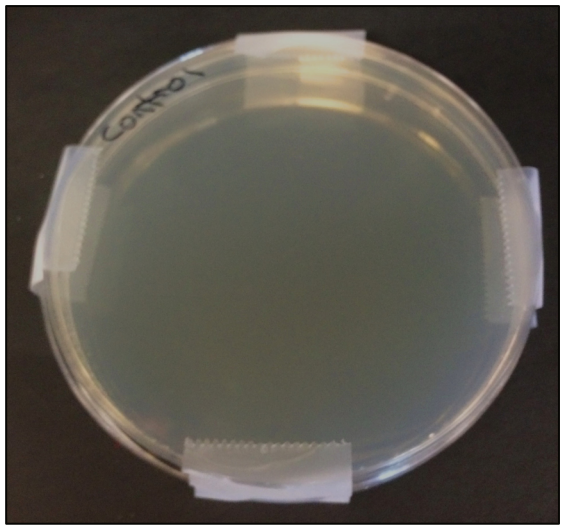
Incubation is the period after the inoculation of an agar plate that microorganisms require for growth and reproduction. Incubation is dependent on certain environmental conditions for optimal growth of different microorganisms.

8.1 Sealing and labelling Petri dishes

After inoculation, agar plates are closed by placing the petri dish lid on the base, and then sealed before incubation. In a school laboratory, sealing the agar plate is conducted to prevent accidental opening of the plate while being incubated under aerobic conditions. Environmental (sometimes referred to as 'wild cultures') agar plates should be kept sealed after inoculation, during incubation and when being examined by students, as the identity and risk group of the environmental microorganisms is unknown.

The recommended method of sealing plates in school laboratories is with four short pieces of sticky tape placed at 12 o'clock, 3 o'clock, 6 o'clock and 9 o'clock to close the two sections of the petri dish. See Figure 1. Complete sealing of the petri dish with sticky tape during growth is not recommended as this creates an anaerobic environment inside the dish that promotes growth of undesirable anaerobic bacteria.[51]

Figure 1. Placement of sticky tape at intervals around the agar plate



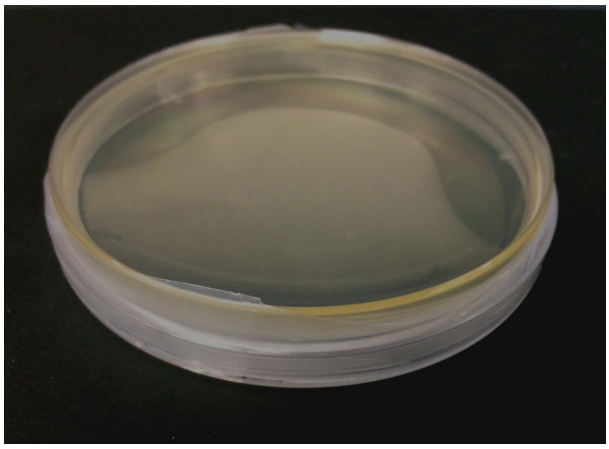
Alternatively, one piece of laboratory sealing film e.g. Parafilm M that is cut no wider than 1cm may be wrapped once around the circumference of the agar plate. The laboratory sealing film should be cut to a length that, when stretched around the agar plate, does not overlap at or beyond its join. See Figure 2. This method prevents leaks of excess fluid from condensation that are potential sources of infection that may come in contact with students or contaminate laboratory areas.

The gas permeability data for Parafilm M indicates that when used as a single layer it will allow sufficient oxygen exchange to promote the growth of aerobic microorganisms and inhibit the growth of potential anaerobes. Wrapping Parafilm around a Petri dish more than once should be avoided and will be sufficient to stop any gas exchange creating an undesirable anaerobic environment.

Parafilm is a laboratory sealing film with unique properties. It is a stretchy, waxy film that is very good at moulding around tops of test tubes, bottles, and flasks and around petri dishes to provide a leak proof seal. It is much more effective than sticky tape.

Each of these methods allows for gas exchange in and out of the agar plate for the growth of aerobic microorganisms.

Figure 2. Placement of laboratory sealing film



Petri dishes should always be labelled on the base containing the media so that if the lid is inadvertently changed the identification of the culture is still known. Information should be written around the perimeter of the plate to avoid covering the colonial growth.

These agar plates are now ready for incubation.

8.2 Environmental conditions required for growth

All living things require certain environmental conditions in which to grow and reproduce. Microorganisms will grow and reproduce in the laboratory when their optimal conditions are met. To promote the growth of Risk Group 1 microbes, certain growth conditions should be met.

In a school science laboratory, there are four main conditions that influence the physical environment for growing Risk Group 1 microorganisms

- Temperature
- Gaseous requirements
- pH
- Osmotic pressure

8.2.1 Temperature

The optimal temperature for growth of microorganisms varies between different species. In laboratory testing of human diseases, incubation temperatures are generally set at 37°C to replicate the temperature of the human body. Microbes grown at these temperatures are generally unknown until further testing has identified them. These microbes may be pathogenic to students and staff.

In the school laboratory, to reduce the growth of pathogens, lower incubation temperatures are used. Agar plates should be incubated between **room temperature (22–25°C) and a maximum of 30°C.** Many cultures suitable for use in schools will grow at room temperature and can be incubated satisfactorily in a cupboard.[52] Refer to Chapter 8.3 Growth of Micro-organisms and time required.

Once grown the cultures may be stored at 4°C. This will slow the growth of any cultures so you can show students a 2–3 day growth if lessons are a week apart.

An incubator may also be used to incubate agar plates as they maintain a constant temperature. Incubators are available from scientific suppliers.

8.2.2 Gaseous atmosphere

An organism that requires oxygen for growth is called 'aerobic'. An organism whose growth cannot occur in the presence of oxygen is 'anaerobic'. An organism that can grow under either aerobic or anaerobic conditions is a facultative anaerobe.[53] In humans, anaerobic bacteria are most commonly found in the gastrointestinal tract and may be pathogenic. The growth of anaerobes and facultative anaerobes should be avoided in schools because of their potential to cause disease in students and staff. They are not classified as Risk Group 1 microorganisms.

Incubating in aerobic conditions, and below human body temperature, reduces the risk of encouraging microorganisms (particularly bacteria) that could be pathogenic to humans.[54] To ensure this occurs, the following practices should be followed:

- Environmental samples should be introduced gently across the surface of the agar with a swab stick, and not penetrate under the agar surface.
- Agar plates should not be sealed in a manner that reduces the exchange of gases in and out of the agar plate.
- Agar plates should not be placed in an airtight container.

8.2.3 pH

The optimum pH for growth varies with different microorganisms. Buffers are usually added to media to prevent sudden pH shifts. Optimal pH conditions for different microorganisms include:

- Bacteria pH 6–8
- Moulds/yeasts pH 5–6
- Protozoa pH 6.7–7.7
- Algae pH 4–8

8.2.4 Osmotic pressure

This generally depends on the concentration of dissolved salt. If the salt concentration outside a cell is too high water is lost from the cell and cell growth is inhibited. Most microbes prefer isotonic solutions where the salt concentration inside the cell is the same as the salt concentration in its environment.

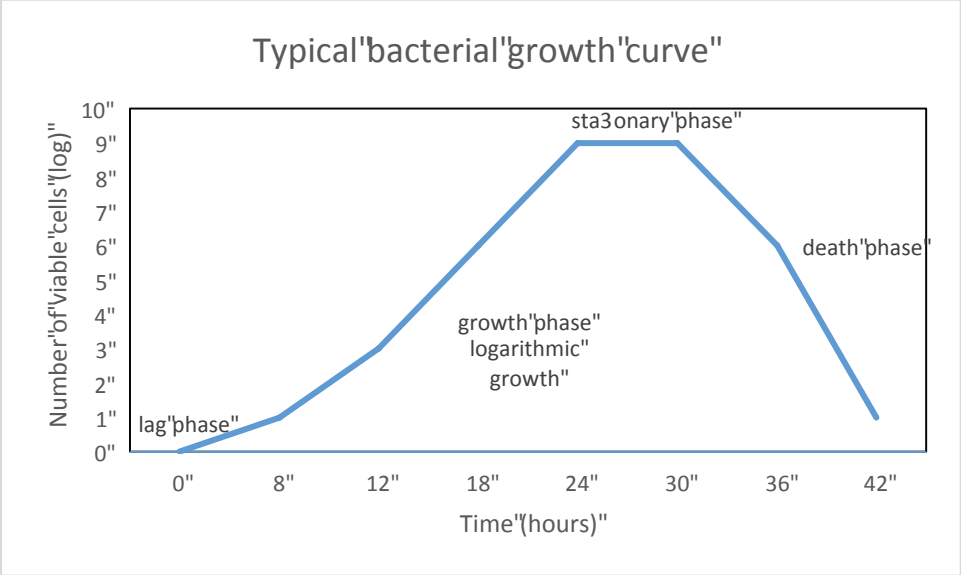
8.3 Growth of microorganisms and time required

When microorganisms are inoculated onto a medium and incubated with optimum conditions for growth, a large increase in the number of cells occurs within a relatively short time.

Optimal time for growing environmental microbes on nutrient agar should be kept to **no greater than 30–36 hours**. After this time, growth and reproduction of microbes slows. Most bacteria multiply by a process called binary fission, some reproduce by budding and some produce spores that develop into a new organism. The following bacterial growth chart shows the typical growth rate of bacteria.

Figure 3: The bacterial growth curve

(Adapted from Todar's Online Textbook of Bacteriology http://textbookofbacteriology.net/growth_3.html)



1. **Lag phase**. Immediately after inoculation of the cells into fresh medium, the population remains temporarily unchanged. Although there is no apparent cell division occurring, the cells are adjusting to their new conditions.
2. **Growth or log phase**. The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation. This is the most rapid (exponential) growth phase.
3. **Stationary phase** Microbe population growth is limited by one of three factors: 1. Exhaustion of available nutrients; 2. Accumulation of inhibitory metabolites or end products; 3. Exhaustion of space. Cell division slows down and the cell numbers stabilise.
4. **Death phase.** If incubation continues after the population reaches the stationary phase, a death phase follows, in which the viable cell population declines.

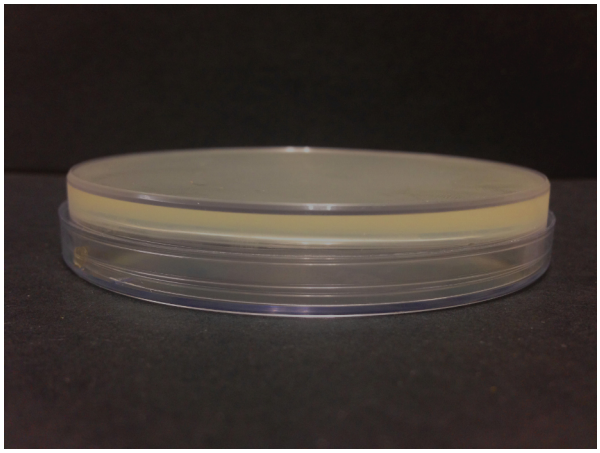
Time of growth can vary between different bacterial species, and different fungal species. Whilst the growth curve above refers to bacteria, the growth of fungi will differ in that a single unit of fungus will grow rapidly in the beginning. The exponential phase will be followed by a plateau, (commonly called the stationary phase) and as the organic nutrients become depleted is followed by a decline phase. These stages are typical of any organism growing in fixed quantities of nutrients, particularly under laboratory conditions.[55] In school laboratories it is not beneficial to incubate microbial samples beyond 36 hours, as available nutrients are exhausted after this time. However, this is also dependent upon the temperature particularly if they are incubated in a cupboard and subject to the room's ambient temperature. (Consider, for example, the difference in ambient temperature between Cairns and Hobart.)

Slime moulds are an exception if a skilled teacher or laboratory technician continues to supplement its growth with oat feeding and maintaining control of the mould by subculturing. See Science ASSIST *SOP: Physarum polycephalum care and use* (Appendix 1).

8.4 Practical considerations for incubation

- Agar plates must be incubated inverted with the medium-containing half (base) of the Petri dish uppermost otherwise condensation will occur on the lid and drip onto the culture. This might cause colonies to spread into each other and increase the risk of spillage of the contaminated liquid. See Figure 4.

Figure 4: Incubating an agar plate with the media uppermost in the Petri dish



- Count the plates out and in again to ensure that all plates are collected at the end of a lesson.
- Plates should be sealed around the whole circumference before viewing to reduce the risk of students opening the plates.

Chapter 9 Observing microbes

Agar plates that have been inoculated and incubated can be stored at 4°C for 4–5 days, or distributed to the class from the incubation area. Storage at 4°C slows growth of microbes and reduces possible bad odours from microbe growth on the agar plate. Plates should be removed from the refrigerator at least 1 hour prior to class to bring them to room temperature to lessen the likelihood of condensation forming inside the plate, making observation of the microbes difficult.

Plates should remain sealed with tape or laboratory-sealing film when distributed to students, and should remain sealed throughout examination. Students and staff should wear PPE (gowns, gloves, safety glasses), and magnifying glasses and plastic rulers should be provided. This equipment should be wiped with disinfectant before and after use.

Place an autoclave bag or oven bag close by to collect plates that have been examined. The plates should be sterilised following examination and before disposal in an autoclave or pressure cooker.

Students and staff should disinfect bench tops and wash hands thoroughly after handling agar plates, prior to progressing to another activity or leaving the laboratory.

9.1 Macroscopic morphology of bacteria and fungi

The term ‘colony morphology’ refers to the visible macroscopic characteristics of a colony. Colonies of different types of bacteria and fungi can be varied in appearance. When sampling from the environment there will likely be many different types of microbes on an agar plate, this is called a mixed culture. Being able to visibly differentiate microbes based on the appearance of their colonies growing on an agar plate is an essential first step in starting the identification process.

Colonies that differ in appearance are typically different bacterial and fungal strains, species, or genera. However, colony morphology is not a reliable way to identify microbes, as many different types of bacteria and fungi have similar colony morphology.[56]

9.1.1 Describing colony morphology

Bacteria. Each distinct single colony represents an individual bacterial cell that has divided repeatedly. Most bacterial colonies appear white, cream or yellow in colour.

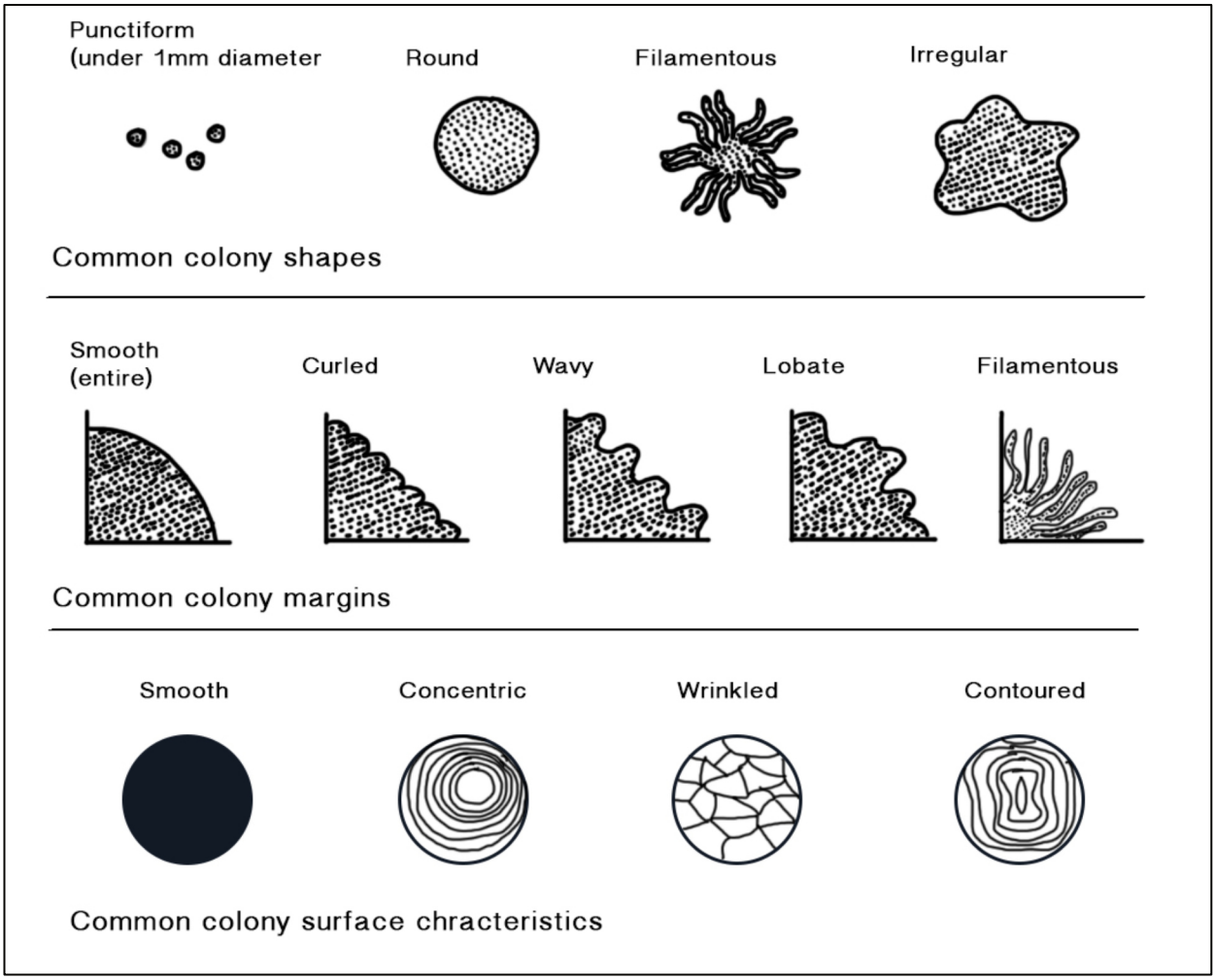
Fungus. Fungal colonies can be divided into yeasts, moulds and dimorphic fungi. **Yeast** colonies can look similar to bacterial colonies. **Moulds** often appear furry and have fuzzy edges. Many turn into a different colour, from the centre outwards. They are identified by the presence of tubular structures known as hyphae. These are masses of individual filaments and look like fuzzy or furry growths. **Dimorphic fungi** are fungi that exist as either yeasts or moulds.[57]

Although bacterial colonies can differ in the details of their appearance, a colony basically looks like a dot growing on the medium. This dot is composed of millions of bacteria. Description of a colony's morphology includes its shape, the margins or edges of the colony, its colour, opacity and surface features. Some colonies are round and smooth; others can have wavy edges and a wrinkled appearance. The morphology of yeast colonies is very similar to bacterial colonies.[58]

Although bacterial and fungal colonies have many characteristics and some can be specific to a species, there are a few basic characteristics that you can identify for all colonies.

- Form: What is the basic shape of the colony? For example, circular, filamentous, etc.
- Size: The diameter of the colony
- Elevation: What is the cross-sectional shape of the colony? Turn the Petri dish on end.
- Margin: What is the magnified shape of the edge of the colony?
- Surface: How does the surface of the colony appear? For example, smooth, glistening, rough, dull (opposite of glistening), rugose (wrinkled), etc.
- Opacity: For example, transparent (clear), opaque, translucent (almost clear, but distorted vision, like looking through frosted glass), iridescent (changing colours in reflected light), etc.
- Colour (pigmentation): For example, white, buff, red, purple, etc. [59]

Figure 5: Colony morphology for bacteria and fungi



9.2 Examining environmental plates

For the method see Science ASSIST *SOP: Microbes are everywhere* (Appendix 1)

9.2.1 Method for examining environmental plates

Provide magnifying glasses and plastic rulers to assist students to examine the colonies. Plates can also be placed under a dissecting microscope for examination.

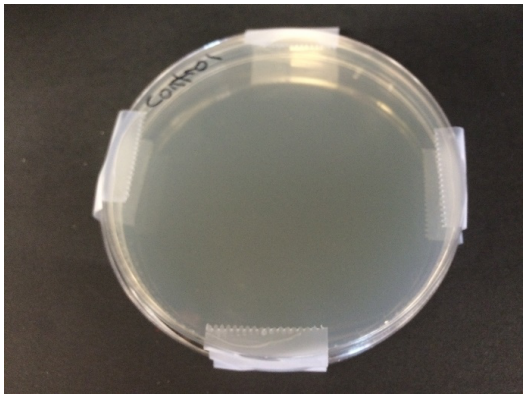
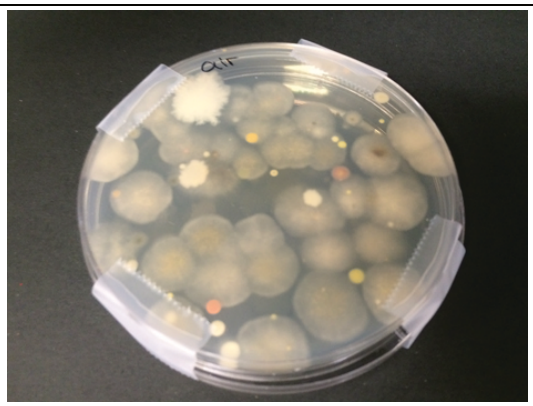
Instructions for students:

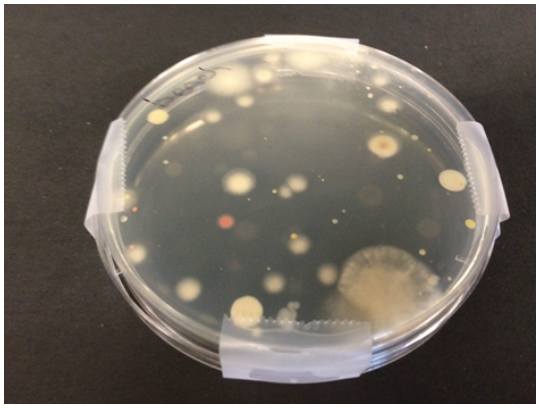
1. Keeping the Petri dish closed, measure the colony diameter in millimetres.
2. Describe the pigmentation (distinguishing between pigmented colonies and those secreting diffusible pigments).

3. Describe the form, elevation, and margin as indicated in Figure 5. Also, indicate whether the colonies are smooth (shiny glistening surface), rough (dull, bumpy, granular, or matte surface), or mucoid (slimy or gummy appearance).
4. Record the opacity of the colonies (transparent, translucent, or opaque).[60]

9.2.2 Example of results of environmental sampling plates

Table 6: Example of results of environmental sampling plates

Plate Number	Sample	Photo and description
1	Control	 No growth
2	Air sample	 Mixed culture. (Not a pure culture). Colonies of bacteria, yeast and fungi grown on nutrient agar. Size of the different colonies varies from approximately 1mm to 12mm in diameter. Pigmentation varies from white, yellow and pink. Most colonies are smooth and are circular in form with an entire margin. There are some irregular shaped colonies with filamentous margins. They have a convex elevation. Most colonies have a smooth surface. There are some colonies with a rough surface. All colonies are opaque in appearance.

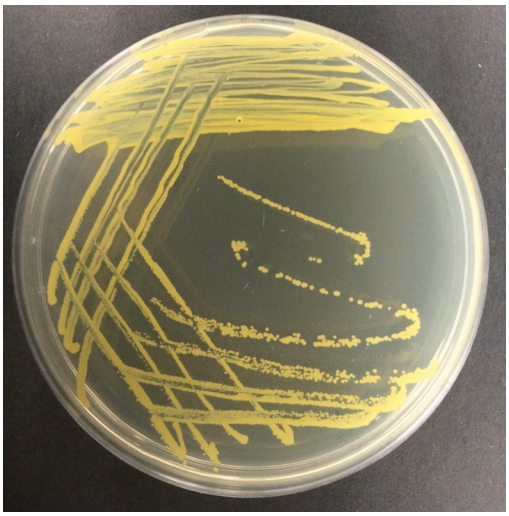
3	Laboratory bench	 Mixed culture (Not a pure culture). Colonies of bacteria, yeast and fungi grown on nutrient agar. Size of the different colonies varies from approximately 1mm to 12mm in diameter. Pigmentation varies from white and yellow. Most colonies are smooth and are circular in form with an entire margin. There are some irregular shaped colonies with filamentous margins. They have a convex elevation. Most colonies have a smooth surface. There are some colonies with a rough surface. All colonies are opaque in appearance.

9.3 Example of results of growth of pure cultures from aseptic inoculation

For methods of inoculating plates with aseptic technique see Chapter 7 Inoculating media, Science ASSIST *SOP: Streak plate inoculation*, *SOP: Preparing a bacterial lawn* (Appendix 1).

Streak plate inoculation

Figure 6: Streak plate inoculation of *Micrococcus luteus* on nutrient agar, incubated for 24 hours at 28°C.



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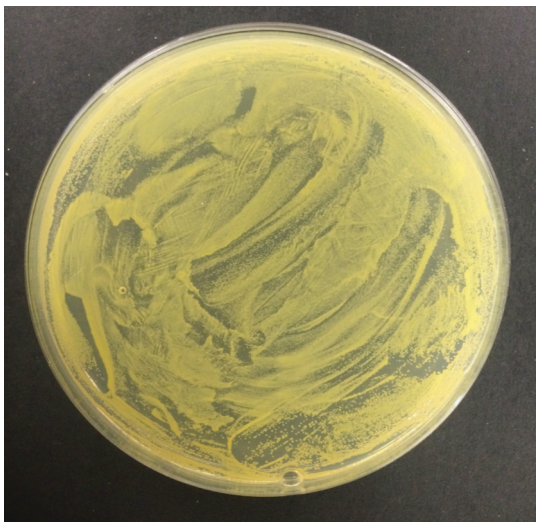
Lawn inoculation

Figure 7: Lawn inoculation of *Micrococcus luteus* on nutrient agar, incubated for 24 hours at 28°C.



9.4 Examination of *Physarum polycephalum*

See Science ASSIST *SOP: Physarum polycephalum care and use* (Appendix 1) for instructions on how to demonstrate this in class.

Physarum polycephalum is a slime mould that grows in dark humid conditions under the bark of decaying trees and amongst leaf litter on the forest floor. *Physarum polycephalum* evolves through several life stages. The plasmodium stage is the stage most likely to be present on the agar plate when fed and housed under optimal conditions. This is the stage that is of most interest to students, as subculturing can take place, and examination and experimentation can occur. The simplest activity in the laboratory is the demonstration of cytoplasmic streaming.

For further information on the life cycle of *Physarum polycephalum* see 'Physarum life cycle', Southern Biological website, http://file.southernbiological.com/Assets/Products/Specimens/Living_Specimens_and_Supplies/Plants_and_Fungi/L2_30-Physarum_slime_mould_culture/L2_30_Physarum_LifeCycle.pdf (Accessed December 2016)

9.5 Determining the zone of inhibition on antibiotic and disinfectant susceptibility plates using the disc diffusion method

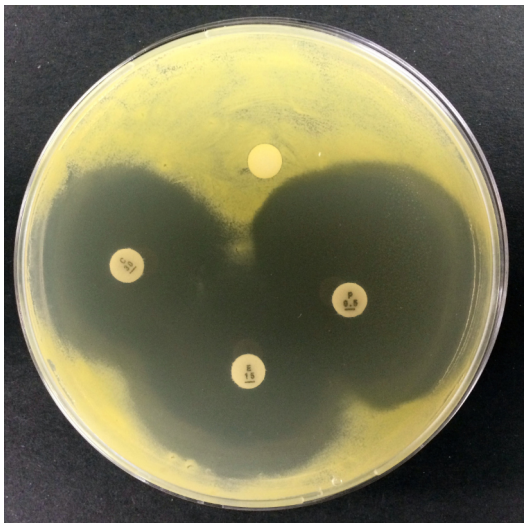
For methods of inoculating plates and introducing discs see Science ASSIST *SOP: Preparing a bacterial lawn* and Science ASSIST *SOP: Susceptibility testing of antiseptics and disinfectants* (Appendix 1)

The susceptibility or sensitivity of a microorganism to antibiotics or disinfectants is a common technique used in pathology, food technology and industrial laboratories. In these tests an antibiotic or disinfectant is incorporated into a paper disc, which is placed on an agar plate containing a lawn culture of the organism to be tested. The antibiotic or disinfectant diffuses into the agar. Sensitivity or resistance is determined by observing zones of inhibition around the discs.

If the organism is sensitive (susceptible) to the agent, then a zone of inhibition (clearing) is observed around the disc. Absence of a zone of inhibition is indicative of resistance. The

effectiveness of the antibiotic or disinfectant is determined by the size of this zone (See Figure 8).

Figure 8: Sample susceptibility test result. *Micrococcus luteus* grown on nutrient agar, incubated for 24 hours at 28°C.
Clockwise from top: control disc, Penicillin 0.5µg, Erythromycin 15µg, Chloramphenicol 30µg.



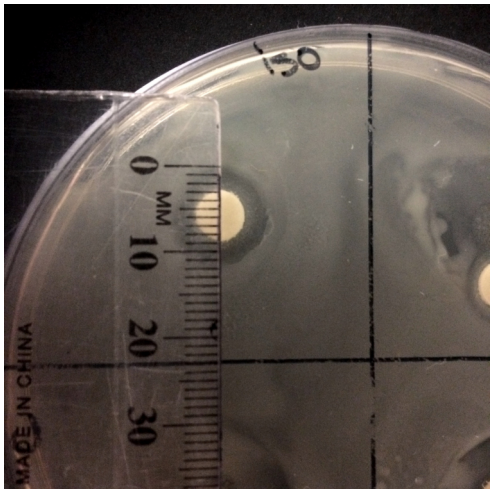
The bigger the area of microorganism-free agar around the disc means the bacteria are more sensitive to the disinfectant. It is expected that there will be no clear area around the control disc, and a larger area around the discs that have been impregnated with an effective disinfectant. This clear area indicates the effectiveness of the disinfectant at killing the microorganism.

9.5.1 Method of measurement

Zones of inhibition are measured using a millimetre ruler. Professional laboratories may have an automated zone reader.

1. Place the agar plate flat on a workbench with the agar side upper most.
2. Place a ruler on the plate with the zero at the edge of microbial growth surrounding one disc.
3. Measure the clear area across the clear zone, including the disc. Record this measurement in millimetres.
4. Repeat for each individual disc.

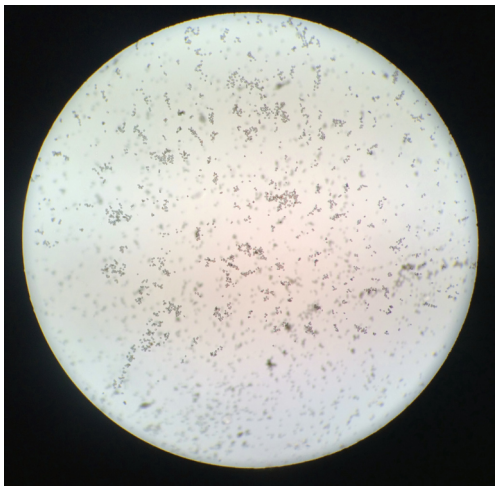
Figure 9: Measuring the zone of inhibition of iso-Propyl alcohol on *Escherichia coli* K–12 grown on a nutrient agar plate, incubated for 24 hours at 28°C.



9.6 Examining mushroom spores

For methods of collecting mushroom spores see Science ASSIST *SOP: Examine mushroom spores* (Appendix 1).

Figure 10: Mushroom spores collected from a Portobello mushroom and examined at 400x magnification.



9.7 Examining pond water

For method of preparing pond water slides see Science ASSIST *SOP: Examining life in pond water* (Appendix 1).

Further information and identification keys can be found at:

- ‘The Aquatic Microfauna’, Australian Waterlife website, http://australianwaterlife.com.au/microfauna.html (Accessed December 2016)
- Maths/Science Nucleus. 2004. *Guide to Identification of Fresh Water Microorganisms*, Maths/Science Nucleus website, http://www.msnucleus.org/watersheds/mission/plankton.pdf
- ‘Pond identification sheet’, Biology Corner website, http://www.biologycorner.com/worksheets/identifypond.html (Accessed December 2016)

9.8 Microscopic examination of a Gram stain

For method of gram staining see Science ASSIST *SOP: Gram stain of a microbial culture smear* (Appendix 1).

The Gram stain is one of the most important stains in the science of microbiology. It divides bacteria into two broad groups. The reaction of bacteria to the Gram stain is a reflection of differences in bacterial cell walls.

- Gram positive – those that retain the primary basic dye and stain purple.
- Gram negative – those that lose the primary dye and stain red with the counterstain.

Bacteria that have been Gram stained are observed microscopically using an oil immersion objective at 1000x magnification. Bacterial cells will appear very small, even at this magnification. Care must be exercised to ensure that artefacts such as dirt on the slide, air bubbles in the immersion oil, undissolved salts contained with the stain and lint from lens tissue are not confused with the microorganism being observed. For additional information on the use of the light microscope see Science ASSIST *SOP: Use and care of the compound light microscope* (Appendix 1).

The expected microscopic morphology and Gram stain result of some Risk Group 1 bacteria is set out in Table 7.

Table 7: Expected Gram stain results

Microorganism	Gram stain	Shape	Description	Diagram (enlarged representation)
Bacillus subtilis	positive	Bacillus Rod shaped	4–10µm long and ~0.25–1µm diameter Singles or in chains	
Escherichia coli	negative	Bacillus Rod shaped	~ 2µm long and ~0.25–1µm diameter Single cells	
Micrococcus luteus	positive	Coccus Spherical	irregular clusters, tetrads or pairs 1.0–1.8µm diameter	 Cocci in pairs  Cocci in tetrads  Cocci in clusters
Staphylococcus epidermidis	positive	Coccus Spherical	0.5–1.5µm diameter cocci usually form in clusters	 Cocci in clusters

Gram stain results are used to plan further investigations to identify bacteria. In professional labs, a suite of tests is carried out to ensure the bacterial sample is correctly identified.

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[1] 'Microbes and Human Life', Life Materials Technologies Ltd website, http://www.life-materials.com/microbes-and-human-life.html (Accessed December 2016)

[2] 'Salmonellosis – Topic overview', WebMD website, http://www.webmd.com/food-recipes/food-poisoning/salmonellosis-topic-overview (Accessed December 2016)

[3] '*Legionella* (Legionnaires' Disease and Pontiac Fever', Centres for Disease Control and Prevention website, http://www.cdc.gov/legionella/about/causes-transmission.html (Accessed December 2016)

[4] 'Staphylococcus infections', U.S. National Library of Medicine, MedlinePlus website. https://www.nlm.nih.gov/medlineplus/staphylococcalinfections.html (21 October 2016)

[5] 'Aspergillosis', Centers for Disease Control and Prevention website https://www.cdc.gov/fungal/diseases/aspergillosis/ (21 January 2016)

[6] 'Candida', The Free Dictionary by Farlex website, http://medical-dictionary.thefreedictionary.com/Candida (Accessed December 2016)

[7] 'Protozoa', Microbe World website, http://www.microbeworld.org/types-of-microbes/protista/protozoa (Accessed December 2016)

[8] 'Prokaryote', Dictionary.com website, http://dictionary.reference.com/browse/Prokaryote (Accessed December 2016)

[9] 'Eukaryote', Dictionary.com website, http://dictionary.reference.com/browse/Eukaryote (Accessed December 2016)

[10] 'Types of microorganisms', Weber University website, http://faculty.weber.edu/coberg/class/2054/Micro2054%20Types%20of%20Microorganisms.htm (Accessed December 2016)

[11] World Health Organisation (WHO). 2004. *Laboratory biosafety manual*. 3rd edition. WHO website, http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf

[12] Australian Standards/NZ Standards. 2010. *AS/NZS 2243. 2010. Safety in laboratories Part 3: 'Microbiological safety and containment'*. Reproduced with permission from SAI Global Ltd under Licence 1407-c117

[13] Australian Standards/NZ Standards. 2010. *AS/NZS 2243. 2010. Safety in laboratories Part 3: 'Microbiological safety and containment'*. Reproduced with permission from SAI Global Ltd under Licence 1407-c117

[14] 'Work Health and Safety – Laboratory Safety', Western Sydney University website, http://www.uws.edu.au/whs/whs/labsafety (Accessed December 2016)

[15] 'Good microbiological laboratory practice', Microbiology online website, http://microbiologyonline.org/teachers/safety-information/good-microbiological-laboratory-practice (Accessed December 2016)

[16] 'Microbiology', University of Sydney website, http://sydney.edu.au/whs/guidelines/biosafety/microbiol.shtml (5 August 2016)

[17] 'Good microbiological laboratory practice', Microbiology online website, http://microbiologyonline.org/teachers/safety-information/good-microbiological-laboratory-practice (Accessed December 2016)

[18] Science ASSIST. 2015. *AIS: Risk Management and risk assessment*, Science ASSIST website, https://assist.asta.edu.au/resource/3079/ais-risk-management-and-risk-assessment?search-id=323f5ba

[19] Centers for Disease Control and Prevention. 2009. *Biosafety in microbiological and biomedical laboratories (BMBL) 5th Edition*, Centers for Disease Control and Prevention website, http://www.cdc.gov/biosafety/publications/bmbl5/

[20] Kuhnert, Peter and Frey, Joachim. nd. *Tools for Safety Assessment Identification and monitoring of Escherichia coli K-12 safety strains',* Centre for Biosafety and Sustainability website, http://www.bats.ch/bats/publikationen/1996-1_e.coli/96-1_e-coli_k12.php (Accessed December 2016)

[21] Microbiology in Schools Advisory Committee (MISAC). 2010. *Suitable and unsuitable micro-organisms,* MISAC website, http://www.misac.org.uk/PDFs/MiSAC_suitable_and_unsuitable_micro-organisms.pdf

[22] Centers for Disease Control and Prevention. 2009. *Biosafety in microbiological and biomedical laboratories (BMBL) 5th Edition*, Centers for Disease Control and Prevention website, http://www.cdc.gov/biosafety/publications/bmbl5/

[23] US Aid ChallengeTB website. nd. *Standard Operating Procedure (SOP) Use of personal protective equipment for culture and drug susceptibility*, http://www.tbcare1.org/publications/toolbox/tools/sops/08_PPE_Culture_DST.doc (Accessed December 2016)

[24] Science ASSIST. 2015. *AIS: School science area security*, Science ASSIST website, http://assist.asta.edu.au/sites/assist.asta.edu.au/files/AIS%20-%20School%20science%20area%20security.pdf

[25] Macquarie University. 2012. *Microbiological Risk Management: A Guide for Researchers*, Macquarie University website, https://staff.mq.edu.au/public/download.jsp?id=78915

[26] 'Work health and safety – Laboratory safety'. University of Western Sydney website. http://www.uws.edu.au/whs/whs/labsafety (Accessed December 2016)

[27] Townsend, David. 2009. *Biological Safety Manual*. Curtin University website, http://healthandsafety.curtin.edu.au/local/docs/2011_BiologicalSafety_Manual.doc

[28] 'B1.2 Personal Protective Equipment', National Health and Medical Research Council website, http://www.nhmrc.gov.au/book/export/html/30058 (5 October 2010)

[29] American Society for Microbiology (ASM). 2012. *Guidelines for Biosafety in Teaching Laboratories*, ASM website, http://www.asm.org/index.php/education-2/22-education/8308-new-version-available-for-comment-guidelines-for-best-biosafety-practices-in-teaching-laboratories

[30] 'Safety glasses', Science ASSIST website, http://assist.asta.edu.au/question/2730/safety-glasses?search-id=39dd940 (April 2015)

[31] 'Decontaminate', Dictionary.com website, http://dictionary.reference.com/browse/decontamination (Accessed December 2016)

[32] 'Sterilization', The Free Dictionary by Farlex website, 'http://www.thefreedictionary.com/sterilization (Accessed December 2016)

[33] Centers for Disease Control and Prevention. 2009. *Biosafety in microbiological and biomedical laboratories (BMBL) 5th Edition*, Centers for Disease Control and Prevention website, http://www.cdc.gov/biosafety/publications/bmbl5/

[34] Biosafety in Microbiological and Biomedical Laboratories. 2009. *Decontamination and Disinfection Appendix B.* Centers for Disease Control and Prevention website http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixB.pdf

[35] Rutala W, Weber, David J, Healthcare Infection Control Practices Advisory Committee. 2008. *Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008.* Centers for Disease Control and Prevention website, www.cdc.gov/hicpac/pdf/guidelines/Disinfection_Nov_2008.pdf

[36] CCH. 2011. 'Disinfection and Decontamination 35–268' in *Australian Laboratory Safety Manual*. CCH Australia Ltd. Subscription required (Accessed December 2016)

[37] AS/NZS 2243. 2010. *Safety in laboratories Part 3: 'Microbiological safety and containment Table F1 Summary of Recommended Applications for Chemical Disinfectants in Microbiological Laboratories'* reproduced with permission from SAI Global Ltd under Licence 1407-c117

[38] Centers for Disease Control and Prevention. 2009. *Biosafety in microbiological and biomedical laboratories (BMBL)* 5th Edition, Centers for Disease Control and Prevention website, http://www.cdc.gov/biosafety/publications/bmbl5/, p327

[39] 'Antiseptics', DermNet New Zealand website, http://www.dermnetnz.org/treatments/antiseptics.html (2005)

[40] University of Kentucky, Department of Biological Safety. nd. *Laboratory Disinfectants*. University of Kentucky website, http://ehs.uky.edu/docs/pdf/bio_laboratory_disinfectants_0001.pdf (Accessed December 2016)

[41] ChlorhexidineFacts.com, http://www.chlorhexidinefacts.com/ (Accessed December 2016)

[42] 'Good microbiological laboratory practice', Microbiology Online website, http://microbiologyonline.org/teachers/safety-information/good-microbiological-laboratory-practice (Accessed December 2016)

[43] Rutala W, Weber, David J, Healthcare Infection Control Practices Advisory Committee. 2008. *Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008.* Centers for Disease Control and Prevention website, www.cdc.gov/hicpac/pdf/guidelines/Disinfection_Nov_2008.pdf

[44] 'Autoclaving disposable Petri dishes', Question to Science ASSIST, Science ASSIST website, https://assist.asta.edu.au/question/4146/autoclaving-disposable-petri-dishes (November 2016)

[45] 'Spill kits', Science ASSIST website, http://assist.asta.edu.au/question/2554/spill-kits (December 2014)

[46] 'Spill kits', Science ASSIST website, http://assist.asta.edu.au/question/2554/spill-kits (December 2014)

[47] 'Nutrient agar: composition, preparation and uses', Microbiology Info website, http://www.microbiologyinfo.com/nutrient-agar-composition-preparation-and-uses/ (Accessed December 2016)

[48] 'All about Agar', Science Buddies website, http://www.sciencebuddies.org/science-fair-projects/project_ideas/MicroBio_Agar.shtml (Accessed December 2016)

[49] CLEAPSS. 2011. *Recipe book,* CLEAPSS website, http://www.cleapss.org.uk. Subscription required. (Accessed December 2016)

[50] Southern Biological. 2006. *Nutrient agar plates,* Southern Biological website, http://file.southernbiological.com/Assets/Products/Kits_and_Equipment/Microbiology_Equipment_and_Supplies/Plates/MED3_30-N_A_Plates/MED3_30_NutrientAgarPlates.pdf

[51] Dr Peta Tilbrook. 2015. *Recommendations for safety guidelines for best practice for school microbiology for Science ASSIST*. Curtin University. WA.

[52] Society for General Microbiology. 2006. *Basic Practical Microbiology – A Manual.* Microbiology Online website, http://www.microbiologyonline.org.uk/file/ca2189fba3b39d24c5a44c1285d0082c.pdf

[53] 'Anaerobic bacteria', University of Maryland Medical Center website, http://umm.edu/health/medical/ency/articles/anaerobic-bacteria#ixzz3jbCECzDB (Accessed December 2016)

[54] 'Incubating and viewing plates', Nuffield Foundation website, http://www.nuffieldfoundation.org/practical-biology/incubating-and-viewing-plates (Accessed December 2016)

[55] 'Growth of Hyphae & Development of Fungi', University of Sydney website, http://bugs.bio.usyd.edu.au/learning/resources/Mycology/Growth_Dev/hyphalGrowth.shtml (June 2004)

[56] 'Bacterial Colony Morphology & Identification of Bacteria', Science Prof Online website, http://www.scienceprofonline.com/microbiology/bacterial-colony-morphology-identification-unknown-bacteria.html (Accessed December 2016)

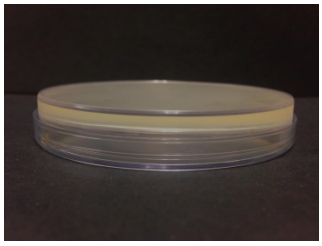
[57] 'Dimorphic Fungi: What are they?', Mold and Bacteria Consulting Laboratories website, http://www.moldbacteria.com/mold/dimorphic-fungi-what-are-they.html (Accessed December 2016)

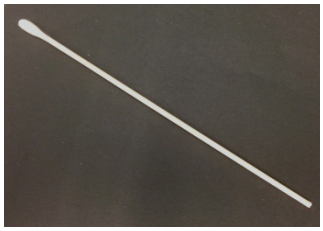
[58] 'Observing fungi in a petri dish', Microbiology Online website, http://www.microbiologyonline.org.uk/teachers/observing-microbes/observing-fungi-in-a-petri-dish (Accessed December 2016)

[59] 'Interpreting plates', Science Buddies website, http://www.sciencebuddies.org/science-fair-projects/project_ideas/MicroBio_Interpreting_Plates.shtml (Accessed December 2016)

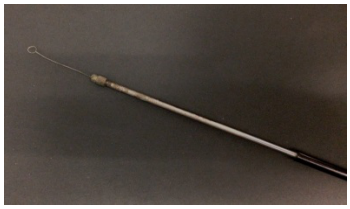
[60] Breakwell, Donald; MacDonald, Bryan; Woolverton, Christopher; Smith, Kyle; Robison, Richard. 2007. *Colony morphology protocol*. American Society for Microbiology website, http://www.microbelibrary.org/component/resource/laboratory-test/3136-colony-morphology-protocol

Glossary of microbiology

Term	Definition/use
aerobic	Growth in the presence of oxygen or requiring oxygen to live
agar media	Consists of a mixture of protein digests (peptone, tryptone) and inorganic salts, hardened by the addition of 1.5% agar, which supports the growth of a wide variety of bacteria
agar plate 	A petri dish containing an agar growth media
anaerobic	An organism not requiring oxygen, or living in the absence of air or free oxygen
antibiotic	A substance such as penicillin that is capable of destroying or weakening certain microorganisms, especially bacteria or fungi, that cause infections or infectious diseases
antiseptic	A substance that inhibits the proliferation of infectious microorganisms on living tissue.
aseptic	Free of microorganisms that cause disease, free from living pathogenic organisms, sterile
autoclave	An apparatus in which steam under pressure effects sterilisation.
autoclave bag 	Commercially available polypropylene bags marked "Autoclave" designed to withstand heat and pressure of autoclave use. Alternatively, an oven bag may be used
bacteria	Microorganisms made up of a single cell that have a cell wall & no distinct nucleus.
Bijou bottle 	A small glass screw-capped bottle of 7mL capacity, used for liquid cultures

Term	Definition/use
bleach (Sodium hypochlorite)	Sodium hypochlorite is a solid white powder, but is more commonly used or purchased dissolved in water. It has antimicrobial activity, as it can react with proteins and DNA of bacteria, as well as breaking down their cell membranes.
broth media 	A nutrient-infused liquid medium used for growing bacteria. Nutrient broth consists of the same ingredients as Nutrient agar, but without agar as a setting agent
chemotaxis	The movement of a microorganism or cell in response to a chemical stimulus
chromogenesis	A bacterium that produces a pigment
colony morphology	A macroscopic description of cells grown on an agar plate. Descriptors include size, surface pigmentation, opacity, and form, elevation and margin taken from an individual colony.
conidia	an asexual spore formed at the tip of a specialized hypha (conidiophore) in fungi such as *Penicillium*
conidiophores	a specialized fungal hypha that produces conidia
contaminated waste bag 	A heavy-duty polyethylene waste bag that is marked with “contaminated waste” for easy identification ensuring correct disposal of waste. Not suitable for use in pressure cookers or autoclaves
contamination	The presence of extraneous material or microorganisms that renders a substance or preparation impure or harmful
cotton swab stick 	A 150mm long wooden applicator stick with a bud of rayon or cotton wool wound around one end. May be purchased sterile and individually wrapped
culture	A growth of microorganisms, viruses, or tissue cells in a specially prepared nutrient medium under supervised conditions

Term	Definition/use
cytoplasmic streaming	The movement of the fluid substance (cytoplasm) within a plant or animal cell. The motion transports nutrients, proteins, and organelles within cells.
decontamination	To make an object or area safe for unprotected personnel by removing, neutralizing, or destroying any harmful substance
dimorphic fungi	Fungi which can exist as either a mould or yeast
disinfect/disinfection	To cleanse something so as to destroy or prevent the growth of disease-carrying microorganisms
disinfectant	Any agent (such as heat, radiation or a chemical) used chiefly on inanimate objects to destroy or inhibit the growth of harmful organisms
endospore	An inactive form that certain bacteria assume under extreme conditions to protect it from damage. Bacteria have been known to remain dormant but alive in the form of endospores for long periods of time
environmental microbe	A microbe that is grown from an unknown source such as those found in environmental sampling. A microbe that has not been identified in the laboratory. Also referred to as a wild microbe
environmental swab	A series of swabs taken from everyday areas in our environment, such as from laboratory benches and door handles, and inoculated onto nutrient agar plates for incubation and examination.
eukaryotic	An organism whose cells contain a nucleus
filamentous	Contains long slender cells or a series of attached cells, as in some algae and fungi
forceps 	An instrument resembling a pair of pincers, used for grasping, manipulating, or extracting. Forceps are generally constructed out of metal, which can be sterilised by autoclaving.
fungi	Any of a wide variety of organisms that reproduce by spores, including the mushrooms, moulds, yeasts and mildews. The spores of most fungi grow a network of slender tubes called hyphae that spread into and feed off dead organic matter or living organisms
Gram stain	A differential staining technique used to classify bacteria into two groups. A bacterial specimen is first stained with crystal violet, then treated with an iodine solution, decolourized with alcohol, and counterstained with safranin. Because of differences in cell wall structure, gram-positive bacteria retain the violet stain whereas gram-negative bacteria do not.
hospital grade disinfectant	Hospital grade disinfectants kill microorganisms, but not necessarily their spores, and should be used on hard, inanimate, non-porous surfaces and semi-critical (contact

Term	Definition/use
	mucus membranes) and non-critical (contact intact skin, environmental surfaces) objects. Hospital grade disinfectants with specific biocidal claims (virucidal, sporicidal, tuberculocidal, fungicidal or other) must be registered on the Australian Register of Therapeutic Goods.
immunocompromised	Having an impaired immune system and therefore incapable of an effective immune response, usually as a result of disease that damages the immune system
incubate	To maintain at a specific favourable temperature and in other conditions promoting development, as cultures of bacteria. The act of keeping an organism, a cell, or cell culture in conditions favourable for growth and development
incubator 	An apparatus in which media inoculated with microorganisms are cultivated at a constant temperature
infection	Invasion of the body by pathogenic microorganisms
infectious	Communicable by infection, as from one person to another
inoculate	To introduce microorganisms into surroundings suited to their growth, as a culture medium
inoculating loop 	A tool usually made of platinum or nichrome wire in which the tip forms a small loop with a diameter of about 5mm, and is used to smear, streak or take an inoculum from a culture of microorganisms
inoculation spreader 	A glass or disposable plastic rod with a bend to look like a hockey stick. Also known as an 'L' shaped spreader or hockey stick spreader. Used in microbiology to create a lawn culture, by spreading liquid culture evenly over an agar plate.
isotonic	A solution containing the same salt concentration as mammalian blood.
margin	Margin or edge describes the borders of a colony

Term	Definition/use
McCartney bottle 	A glass screw-capped bottle of 28mL capacity, used for solid and liquid cultures
media - growth, culture	A substance, such as agar, in which bacteria or other microorganisms are grown for scientific purposes
microbial aerosol	A fine mist of water droplets containing microorganisms
microbiology	The branch of biology dealing with the structure, function, uses, and modes of existence of microscopic organisms
microorganism	Any organism too small to be viewed by the unaided eye, as bacteria, protozoa, and some fungi and algae
molten agar	Molten agar is agar that is heated to a temperature of above 55°C, where it is in a liquid form. The agar sets at a temperature of 40°C
mordant	Used in staining bacteria. A substance that combines with a stain to fix colour in a material.
morphology	The branch of biology dealing with the size, shape and structure of organisms without consideration of function
mould	Any of various fungi that often form a fuzzy growth (called a mycelium) on the surface of organic matter. Some moulds cause food to spoil, but others are beneficial, such as those used to make certain cheeses and those from which antibiotics like penicillin are developed
nutrient agar	A simple medium that consists of a specific concentration of protein (beef extract, Peptone yeast extract) and Sodium chloride, hardened by the addition of 1.5% agar, which supports the growth of a wide variety of bacteria. Not all bacteria can grow on nutrient agar, some find it too rich and others find it deficient. Nutrient agar does not selectively grow pathogenic bacteria
organism	An individual form of life that is capable of growing, metabolizing nutrients, and usually reproducing
parasitic	Caused by a parasite, which is an organism that lives on or in an organism of another species, known as the host, from the body of which it obtains nutriment
pathogen	Any disease-producing agent or microorganism.
pathogenic	Capable of producing disease
pathogenicity	The disease-causing capacity of a pathogen

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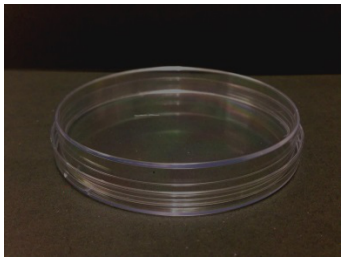
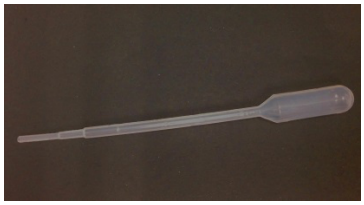
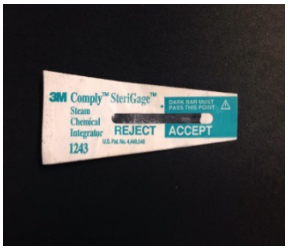
Term	Definition/use
Petri dish  !	A shallow, circular, glass or plastic dish with a loose-fitting cover over the top and sides, used for culturing bacteria and other microorganisms
phototaxis	Response by movement of an organism toward or away from a source of light, response
Physarum polycephalum	*Physarum polycephalum* is a slime mould that inhabits shady, cool, moist areas, such as decaying leaves and logs. It is typically yellow in colour, and eats fungal spores, bacteria, and other microbes.
plain agar	Non-nutrient agar consisting of agar (as a setting agent) and water. Suitable for culture and growth of Physarum polycephalum
plasmodium	A mass of protoplasm having many cell nuclei but not divided into separate cells it is formed by the combination of many amoeba-like cells and is characteristic of the active, feeding phase of certain slime moulds
plastic dropping pipette 	Plastic transfer pipette. 1mL size is ideal for microbiology
PPE	Personal protective equipment: clothing and equipment used to ensure personal safety in the workplace
pressure cooker	A reinforced pot, usually of steel or aluminium which heats to above boiling point by steam maintained under pressure
prokaryotic	Any of a wide variety of one-celled organisms that lack a distinct cell nucleus and their DNA is not organised into chromosomes. They also lack the internal structures bound by membranes called organelles, such as mitochondria
protozoa	Any of a large group of one-celled organisms (called protists) that live in water or as parasites. Many protozoans move about by means of appendages known as cilia or flagella. Protozoans include the amoebas, flagellates, foraminiferans, and ciliates
pure culture	A laboratory microbiological culture containing a single species of organism
risk assessment	An estimate of likelihood of adverse effects that may result from exposure to certain health hazards in the environment

Risk group 1	World Health Organisation classification of microorganisms based on their relative risk. Risk group 1 microorganisms are not associated with disease in a healthy adult
risk management	The techniques of assessing, minimizing, and preventing accidental injury or illness through the use of safety measures
smear	To spread a sample of a microorganism on a glass slide in preparation for staining
sporangia	A single-celled or many-celled structure in which spores are produced, especially in fungi, algae, mosses, and ferns.
sporangiophore	a structure or stalk that bears one or more sporangia
spores	The dormant stage of certain microbial cells. They have thick walls and are able to resist and survive unfavourable environmental conditions.
sporicidal	Chemistry of a substance or product that kills spores.
sporulate	To produce or release spores
sterile	Free from disease-causing microorganisms or live bacteria
sterile paper disc	Sterile blank disc of paper used in susceptibility tests saturated with an antiseptic or disinfectant. Available from biological suppliers. May be purchased impregnated with an antibiotic.
sterilisation	The destruction of all living microorganisms including spores in or on a given environment, such as a laboratory bench, in order to prevent the spread of infection. Using heat, radiation, or chemical agents usually does this.
sterilisation indication strips ! 	or sterility compliance strip. Commercially available strips placed in an autoclave or pressure cooker with items to be sterilised. The strip indicates if the correct time, temperature and pressure have been reached during the run time.
streak	The propagation of microorganisms or living tissue conducive to their growth. A culture is drawn across agar in a zigzag fashion with a wire loop carrying the inoculum.
transmission	The conveyance of disease from one person to another.
viable	Capable of living, developing, or germinating under favourable conditions.
wild microbe	A microbe that is grown from an unknown source such as those found in environmental sampling. A microbe that has not been identified in the laboratory. Also referred to as environmental microbe.
yeast	Any of various one-celled fungi that reproduce by budding and can cause the fermentation of carbohydrates, producing carbon dioxide and ethanol. Some varieties of yeast are used in making beer and wine, other yeasts are pathogenic to humans.
zone of inhibition	The clear region around the paper disc saturated with antimicrobial agent on the agar surface.

Glossary references

‘All about agar’, Science Buddies website, http://www.sciencebuddies.org/science-fair-projects/project_ideas/MicroBio_Agar.shtml

‘Cultivation Media for Bacteria’, Michigan State University website, http://learn.chm.msu.edu/vibl/content/differential/

‘Cytoplasmic streaming’, Encyclopaedia Britannica website, http://www.britannica.com/science/cytoplasmic-streaming

Dictionary.com, Dictionary.com

Encyclopaedia Britannica http://www.britannica.com/

‘Everyday Compounds: Sodium hypochlorite’, Compound Interest, http://www.compoundchem.com/2014/07/06/sodium-hypochlorite-bleach-swimming-pools-cleaning-products/

‘Identifying and using hospital grade disinfectants’, CleanLink website, http://www.cleanlink.com/hs/article.aspx?id=16318

Oxfordreference.com. www.oxfordreference.com

‘Physarum polycephalum’, Encyclopaedia of Life website, http://eol.org/pages/1002810/overview

‘Spill response accessories’, Seton Australia website, http://www.seton.net.au/media/technicalinformation/MB219.pdf

The Free Dictionary. www.thefreedictionary.com

ATTACHMENT 1

Standard Operating Procedures (SOPs)

The Standard Operating Procedures in Attachment 1 were removed in December 2021. They were updated and made available to schools which had a subscription to Science ASSIST.

See the Australian Science Teachers Association website for information about accessing Science ASSIST resources.

ATTACHMENT 2

ASSIST Information Sheets (AIS)

1. Decontaminate microbiological equipment
2. Microwave, pressure cooker or autoclave? Recommendations for best practice of sterilising agar
3. Preparing sterile equipment for microbiology

ASSIST AUSTRALIAN SCHOOL SCIENCE INFORMATION SUPPORT FOR TEACHERS AND TECHNICIANS

ASSIST INFORMATION SHEET:

Decontaminating microbiological equipment

Prompt and thorough decontamination of equipment used in microbiological activities is vital to protect staff, students and facilities from microbiological contamination. These procedures are suitable for the decontamination of Risk Group 1 microbes used in a Physical Containment level 1 laboratory.

Decontamination processes should commence during and immediately after a microbiology activity. Processes such as disposing of disposable items in bleach solution and wiping benches with 70% v/v ethanol after an activity is completed are implemented to contain any microbes and stop the transmission to students and staff in the following lab sessions.

Provide containers with relevant solutions and contaminated waste bags e.g. autoclave bags or oven bags for students to segregate and dispose of equipment and cultures. Each receptacle should be labelled with its function. Ensure these are removed from the laboratory at the end of the activity.

Procedure for using an autoclavable biohazard or oven bag for sterilising microbiological waste:

- **Loosely pack microbiological waste including agar plates into bags to no more than 2/3 full**. This will ensure that the steam during sterilisation will penetrate the entire load. Bags that are tightly filled to capacity will not allow effective steam penetration and the contents will not be sterilised even if all sterilisation parameters are met.
- **Make sure there are no sharp objects present** that may puncture the bag.
- **Loosely tape shut the bag leaving an opening of about 5–6cm** to allow good steam penetration. This can be done with autoclave tape or a rubber band. Never tightly close the bags as they are impervious to steam and therefore the temperature of the inside of the bag will not be sufficient for sterilisation.
- It is advisable to **place the bag into a secondary container** within the steriliser to prevent any leakage into the steriliser should the bag rupture. The container must be able to withstand the autoclaving conditions.
- **Do not overload the steriliser** with too many bags as this may block steam circulation.
- **Use a sterility compliance strip** to indicate if the correct time, temperature and pressure have been reached during the run time. These are available from scientific suppliers.
- **Sterilise at 15psi, 121^{o}C for 15–20 minutes.**
- After sterilisation has been verified, the autoclave or oven bag containing waste items should be **disposed of by placing it into a sturdy garbage bag which is sealed for immediate disposal in industrial bins.**
- **Wear heat protective gloves** when removing waste from the steriliser.

See table over the page for suggested decontamination techniques.

References

Society for General Microbiology. 2006. *Basic Practical Microbiology – A Manual.* Microbiology Online website, http://www.microbiologyonline.org.uk/file/ca2189fba3b39d24c5a44c1285d0082c.pdf

CONTAMINATED Item	Suggested decontamination technique
Inoculated agar plates – plastic	Pack unopened plates loosely in an autoclave bag, leaving an opening of about 5–6cm to allow good steam penetration. Autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.
Inoculated culture broth in McCartney or Bijou bottles.	Make sure that the lids are loose. Autoclave at 121°C, 15psi for 15–20mins. Empty contents in the sink with copious amounts of water. Wash in warm soapy water, rinse well and dry. *Resterilise:* Re-autoclave loosely lidded container. Store in a clean closed container.
Inoculated water in glass bottle	Make sure that the lids are loose. Autoclave at 121°C, 15psi for 15–20mins. Empty contents in sink. Wash in warm water and dry. *Resterilise:* Re-autoclave loosely lidded container. Store in a clean closed container.
Plastic dropping pipettes	1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in the general waste.
Used swabs	1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours or. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15-20 mins. When cool, place unopened autoclave bag in a garbage bag and dispose in the general waste.
Sterile 'L' spreader	1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours, or 2) Place directly into an autoclave resistant container and cover with foil or place into an autoclave/oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15-20 mins. 3) After sterilisation, wash in warm soapy water, rinse and dry. *Resterilise:* Wrap in foil and sterilise in an autoclave or hot air oven. Store until required for re-use.
Sterile forceps	Carefully place into an autoclave resistant container such as a large test tube, cover with foil and autoclave. Wash in warm soapy water, rinse and dry. *Resterilise:* Wrap in foil or place inside a clean test tube, cover opening with foil and autoclave, Store until required for re-use.
Test tubes	Autoclave at 121°C, 15psi for 15–20mins. Empty contents in sink. Wash in warm soapy water, rinse and dry. *Resterilise:* Plug with non-absorbent cotton wool and autoclave or cover the opening of the test tubes with foil and sterilise in a hot air oven or autoclave. Store in a clean closed container.

CONTAMINATED Item	Suggested decontamination technique
Inoculating loop	Flame to red heat carefully in the blue flame of the Bunsen burner to prevent the transmission of aerosols. Cool and reuse immediately. Alternatively, if using disposable inoculating loops, 1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours.. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.
Susceptibility discs Mastrings (set of 6 or more antibiotic discs joined together)	Susceptibility discs and Mastrings should remain on the agar plate after examination. The agar plate remains closed. Pack unopened plates loosely in an autoclave bag and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.
Paper towel exposed to contaminated areas Used disposable aprons/lab coats Used gloves	If not soaked in bleach or alcohol, sterilise in an autoclave or pressure cooker. If soaked leave for the recommended time and then dispose of into the general waste. An autoclave or oven bag should be placed in the laboratory for students to place these waste items directly into the bag. Do not overfill the bag. Leave an opening of about 5–6cm to allow good steam penetration and sterilise for 15–20 min at 121°C and 15psi. Place the unopened autoclave bag into a sturdy garbage bag and seal for immediate disposal in an industrial bin.
Laboratory benches Plastic containers used for storage and distribution of equipment Any other hard surface	Dilute disinfectant in fresh water according to the manufacturer's instructions. Use in a spray bottle. Dilute ethanol to 70% in fresh water, use in a wash bottle. Ethanol is flammable. Apply liberally to laboratory bench or other hard surface to be decontaminated. Wipe lightly with paper towel. Allow the residual to air dry.

ASSIST INFORMATION SHEET:

Preparing sterile equipment for microbiology

Equipment used in microbiology should be sterile before using. This enables aseptic techniques to be used when transferring microorganisms for inoculation, sampling environmental areas, adding susceptibility discs to agar plates and Gram staining.

This equipment should be prepared before the class activity and stored in clean, lidded containers.

Equipment such as hockey stick spreaders, inoculating loops and sterile swab sticks can be purchased as single-use items from commercial scientific suppliers if the school budget allows or it is more time effective to do so.

In-house preparation of sterile items is cost effective to schools as some pieces of equipment can be repeatedly recycled. Care should be taken with ethanol as it is a flammable substance and should not be used near a naked flame.

Considerations:

- Sterilisation of equipment should be performed in a draught-free area.
- Items to be sterilised should be clean and dry, metal forceps should not be rusty, glass items should not have chips or cracks.
- Consult the planned activity or activities prior to sterilising items to ensure there is the required number of items available during the activity.
- Ensure the bench area for this purpose has been decontaminated with 70% ethanol prior to commencing.
- Soaking items in a container of 70% (v/v) ethanol for 10 minutes, disinfects/decontaminates, but does not sterilise items. Alcohols are not sporicidal.
- Aluminium foil or greaseproof paper may be used to wrap sterile items.
- Sterile items can be stored in a large lidded plastic container that has been decontaminated with ethanol and paper towel.
- **Glassware and metal instruments can be wrapped in aluminium foil and sterilised using dry heat in an oven at 160°C for 2–3 hours.**
- **All sterilising processes using an autoclave/steriliser or pressure cooker should be at 121°C for 15–20 minutes at 15psi (pounds per square inch of pressure).**
- Professional microbiologists and higher education providers promote the sterilisation technique of ‘flaming’ hockey stick spreaders and forceps prior to using by dipping in 70% ethanol and igniting it in the Bunsen flame. Incorrect techniques can encourage microbial aerosol transmission and risk the ethanol catching on fire. **Science ASSIST does not recommend this practice in the school setting, but instead recommends sterilising these items in an autoclave or an oven.**

Item	Suggested sterilising technique	Alternative technique
Sterile plastic Petri dishes	Purchase sterile, leave wrapped in original packaging until required. (Do not autoclave prior to use. Plates do not retain shape when autoclaved.)	
Sterile glass Petri dishes	Wrap glass Petri dishes in greaseproof paper or aluminium foil and sterilise in an autoclave	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2–3 hours
Nutrient agar plates	Prepare agar solution according to the manufacturer's instructions,!autoclave in a heat-safe bottle with lids loose and pour plates when temperature of sterile agar is ~50^0C using aseptic technique. When set, wrap in plastic wrap. Store at 4°C until required.!See ASSIST SOP: Preparing agar plates	Purchase prepared and sterile from a biological supplier
Nutrient broth	Prepare broth solution according to the manufacturer's instructions. Aliquot ~15mL into McCartney bottles (28mL capacity) keep lids loose. Autoclave. When cool tighten lids and store at 4°C until required.	Purchase prepared and sterile from a biological supplier
Sterile water	Aliquot 2mL into Bijou bottles (7mL capacity) keep lids loose. Autoclave. When cool tighten lids and store at 4°C until required.	
Sterile plastic dropping pipettes	Purchase single-use pipettes from commercial scientific, biological or medical suppliers.	
Sterile swab stick	Purchase sterile, leave wrapped in original packaging until required.	Autoclave cotton buds in foil covered beaker.
Sterile 'L' spreader	Wrap in aluminium foil and autoclave. Store until required.	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2-3 hours.
Sterile forceps	Wrap in aluminium foil or place inside a clean test tube, cover opening with aluminium foil and autoclave. Store until required.	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2-3 hours.
Sterile test tubes/ conical flasks	Cover opening with foil or plug with non-absorbent cotton wool. Autoclave.	Cover opening with aluminium foil. Sterilise using dry heat in an oven at 160°C for 2–3 hours
Inoculating loop	Flame to red heat in the blue flame of the Bunsen burner.	Purchase sterile disposable inoculating loops, leave wrapped in original packaging until required.

References

U.S. Department of Health and Human Services. 2009. *Biosafety in Microbiological and Biomedical Laboratories.* 5th edition. Centers for Disease Control and Prevention website, http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

Society for General Microbiology. 2006. *Basic Practical Microbiology – A Manual.* Microbiology Online website, http://www.microbiologyonline.org.uk/file/ca2189fba3b39d24c5a44c1285d0082c.pdf

https://www.cdc.gov/HICPAC/Disinfection_Sterilization/13_10otherSterilizationMethods.html

ASSIST AUSTRALIAN SCHOOL SCIENCE INFORMATION SUPPORT FOR TEACHERS AND TECHNICIANS

ASSIST INFORMATION SHEET:

Microwave, pressure cooker or Autoclave? Recommendations for best practice of sterilising agar.

Preparation of agar plates is central to effectively and economically expose our students to the wonderful world of micro-organisms. School science labs have long questioned the best physical method of control to sterilise agar prior to pouring into Petri dishes. Methods used for sterilizing include pressure cookers, sterilisers or autoclaves. Most schools have limited financial resources available, and sourcing processes and equipment that are cost effective is a priority. So which method should you choose and why?

Sterility of processes and equipment forms the very essence of good microbiological laboratory practice and the teaching of these methods to students. This includes the preparation and presentation of agar plates and other equipment used either before, during or after the practical has been run. Teaching aseptic technique to students can be part of the unit of learning; however can be a difficult process to master for first timers. Outcomes of student practicals may be misleading or incorrect if external contaminates are introduced at any stage prior to or during inoculation. Unfortunately, this is often not evident until much time and effort by the student and lab technician has passed and the results are interpreted. Ascertaining the source of any contaminant can be difficult and inconclusive.

While many types of agar plates are available commercially, many laboratory technicians prepare plates in-house thus reducing the cost to schools. Although it can be time consuming, many schools find the cost benefit far outweighs time spent. Many school science preparation areas are used for many disciplines of science and are not usually specific to microbiology or necessarily a 'clean' area. Schools may be permitted by their state jurisdictions to use micro-organisms from Risk Group 1* and/or culture environmental samples. It is impossible to predict what, if any, further contaminants may be introduced to an agar plate during a practical session. Hence the need to 'get it right' by initial effective sterilisation of agar to ensure plates distributed to students do not contain any contaminants.

Universally, micro-organisms are ubiquitous. Effective sterilisation of a liquid such as agar is achieved when all viable organisms are eliminated[1]. The most effective and suitable method of sterilising agar is by using moist heat in the form of steam under pressure i.e. 121^{o}C for 15 minutes at 15 pounds per square inch (psi). This method will denature & coagulate enzymes and other cell constituents in the bacterial cell. Sterilization can be guaranteed only when these parameters are reached.

Sterilisation of agar and plates is usually done in an autoclave or a commercially available pressure cooker with a gauge and the capacity to reach 15 psi, which provides these conditions. Microwave ovens will not sterilise as they do not provide these conditions and therefore are not a suitable alternative to a pressure cooker or autoclave. Water boils at 100^{o}C at atmospheric pressure, but if pressure is raised, the temperature at which the water boils also increases. In an autoclave or pressure cooker the water is boiled in a closed chamber. As the pressure rises, the

boiling point of water also raises. At a pressure of 15 psi inside the autoclave, the temperature is said to be 121°C. Exposure of articles to this temperature for 15 minutes sterilises them.[2]

Due to the action of a microwave oven, micro-organisms will not be killed. Microwaves penetrate unevenly and there are also 'hot spots' caused by wave interference. The whole heating process is different because you are 'exciting atoms' rather than 'conducting heat'.[3] The heat and pressure required to effectively sterilise agar will be insufficient and cannot be maintained for the required period of time. The agar will boil over before any of the required parameters are reached.

As laboratory technicians, we are constantly on the lookout for more efficient ways of finding good quality relevant resources for our students and teachers within budgetary constraints. Sourcing equipment such as a pressure cooker or autoclave is important to ensure the validity of student results and is imperative for microbiological safety.

***WHO Risk Group 1** (no or low individual and community risk). A micro-organism that is unlikely to cause human disease or animal disease (AS 2243.3)

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[1] Todar, Kenneth 2008, 'Control of Microbial Growth', Todar's Online Textbook of Bacteriology http://textbookofbacteriology.net/control_1.html (Accessed 01/04/2014)

[2] Rao, Sridhar 2008 'Sterilization and Disinfection', Department of Microbiology, JJMMC, Davangere www.microrao.com/micronotes/sterilization.pdf (Accessed 01/04/2014)

[3] Brain, Marshall 'How microwave cooking works', howstuffworks.com, http://home.howstuffworks.com/microwave2.htm (Accessed 01/04/2014)

ATTACHMENT 3

Laboratory rules – Microbiology

Laboratory rules – Microbiology

- Science ASSIST strongly recommends that all science rooms be locked unless a teacher or other authorised person is present.
- Students must be supervised at all times in the laboratory. Reckless or irresponsible behaviour will not be tolerated
- Eating, drinking, smoking, and shaving and the application of cosmetics are prohibited.
- Storage of food and drink in the laboratory refrigerators is prohibited.
- Work benches must be decontaminated prior to starting work, following spills and when work is completed.
- Precautions must be taken to ensure reading and writing materials do not become contaminated.
- No equipment or materials are to be removed from the laboratory.
- Appropriate Personal Protective Equipment (PPE) must be worn at all times in the laboratory. This includes:
    - Properly fastened laboratory coat or disposal apron
    - Non-slip shoes that cover the toes, upper surface of the foot and the heel.
    - Approved protective eyewear must be worn
    - Appropriate gloves should be worn to suit the particular application
    - Long hair must be tied back
- Laboratory coats must be removed before leaving the laboratory
- Hands must be thoroughly washed at the completion of a task or before leaving the laboratory.
- Inform the teacher or laboratory technician immediately in the event of a spill or breakage.
- Report all accidents, hazards, incidents or injuries to your teacher.
- Do not commence work unless a site-specific risk assessment is completed, and control measures are implemented.
- All microbiological samples should be regarded as potentially hazardous or infectious. Safe handling procedures must be implemented for all hazardous substances or procedures
- A laboratory safety induction must be completed before undertaking any work in a laboratory.
- Cultures must be clearly identified, dated and appropriately stored.
- Care must be taken to minimise the production of aerosols.
- Care must be taken to prevent the dissemination of material while flaming a wire loop.
- Mouth pipetting is prohibited.
- Laboratory waste must be decontaminated prior to disposal. Wastes must not be poured down sinks or drains.
- Your work space should be left clean and tidy at the end of the practical session. Bench surface should be decontaminated before leaving the lab.

Science ASSIST. 2015. *AIS: School science area security*. Science ASSIST website http://assist.asta.edu.au/sites/assist.asta.edu.au/files/AIS%20-%20School%20science%20area%20security.pdf

Western Sydney University Work Health and Safety Unit. 2010. *Laboratory Safety Guidelines*. Western Sydney University website, http://www.uws.edu.au/whs/whs/labsafety