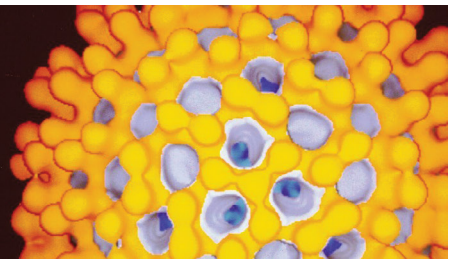
MiSAC
MICROBIOLOGY IN SCHOOLS ADVISORY COMMITTEE
Promoting microbiology in schools and colleges since 1969



# Safety guidelines

The nature of the growth, reproductive capacity and biochemistry of many microorganisms makes them of great economic, social and medical importance. The fundamental rules of personal, public and domestic hygiene rely on an understanding of the characteristics of these organisms. Microorganisms possess many obliging features that make them ideal subjects for safe practical exercises in schools. Unlike many organisms, they do not necessarily have to be maintained over long periods and do not have to be fed and watered at weekends! Staff in schools and colleges should be in no doubt of the considerable educational value of thoughtful, practical microbiological work and of the need for every pupil to possess a basic knowledge of the biology of these organisms.

## Levels of work

Work in microbiology and biotechnology in schools is categorised into three levels which are described in outline below. Although appropriate for use in schools, these levels are not the same as 'levels of containment' used by professional microbiologists. Further detailed guidance for work at these levels is provided in the table Microbiology, overleaf.

- **Level 1 (L1)**: work with organisms which have little, if any, known risk and which can be carried out by teachers with no specialist training. The organisms will normally be observed in the closed (but not sealed) containers in which they were grown.
- **Level 2 (L2)**: work where there are some risks of growing harmful microbes but these are minimised by a careful choice of organisms or sources of organisms and by culturing in closed containers which are taped before examination and remain unopened unless the cultures within have been killed. Once a culture, prepared by pupils, has been grown, subculturing or transfer of organisms from one medium to another is not normally attempted. L2 work can be carried out with pupils between the ages of 11 and 16 years (KS 3 & 4; S1/2 & S3/4 in Scotland) and by science teachers who may require supervision and training, which can be provided through a short in-service course or in school by a knowledgeable biology teacher or technician.
- **Level 3 (L3)**: work where cultures of known microbes are regularly subcultured or transferred. Class work is normally confined to students over the age of 16 and institutions where facilities are appropriate. Teachers should be thoroughly trained and skilled in aseptic technique. This is a higher level of training than required for L2 work. Non-specialist teachers should not carry out or supervise this work.

**Preparation of resources for microbiology work beyond Level 1 will always be at Level 3, and require appropriate expertise and facilities.**

## General safety considerations

Risk assessment in microbiological work is of fundamental importance. For further guidance, refer to the accompanying pdf document *Assessing the risks in microbiology* in the health & safety section of the MiSAC web site.

A significant hazard associated with work in microbiology or biotechnology is the generation of microbial aerosols, where fine droplets of liquid containing cells and/or spores of microbes are released into the air. Aerosols can be formed whenever liquid surfaces are disrupted or material is crushed or ground. The particles are so small that they are easily carried by air currents and can be inhaled into the lungs. Many of the safety measures detailed below are designed to minimise the risk of aerosol formation.

Microbiology and biotechnology share many safety requirements. However, a major difference is one of scale with a corresponding increase of risk when handling larger volumes of microorganisms grown in liquid media.

Before work with microbes is started, pupils should wash their hands, preferably with soap and water, or use an antimicrobial gel, (except for L2 & 3 work investigating microbes on unwashed hands). Any cuts should be covered with waterproof plasters. After working with microbes, hands must always be washed with soap and water.


Microbiology in Schools Advisory Committee, c/o NCBE, University of Reading, 2 Earley Gate, Reading RG6 6AU
E: *microbe@misac.org.uk* W: *misac.org.uk*

# Microbiology

| Source of hazard(s) | Guidance |
|---|---|
| **Organisms** | L1 Limited to:<br>• algae, yeasts, moulds and bacteria used for culinary purposes,<br>• commonly-occurring bacteria and fungi where they grow naturally on decaying vegetable material, and<br>• microbes from ponds and rain water.<br>L2 Care in the choice of suitable cultures (see the accompanying pdf document *Suitable and unsuitable microorganisms*) must be taken by obtaining them from reputable specialist suppliers which would include culture collections and, for live yeast, reputable bakers and health food shops. Where possible, organisms with unusual growth requirements, e.g. high salt, low pH, low temperature, should be chosen but these may not grow well on standard media.<br>Organisms may be cultured from the environment but not from environments which are likely to contain harmful organisms, for example, toilets or body surfaces other than fingers or hands. Containers of environmental cultures, once they have been incubated, must then be sealed before examination.<br>L3 Known cultures from reputable specialist suppliers. Organisms may be cultured from the environment or from some human body surfaces if the work is essential for the course and if cultures are not opened by students. Teachers wishing to use organisms at L2 and L3, not listed as minimum risk, must have had suitable training in microbiological techniques and should consult an appropriate advisory body[1]. Proficiency in aseptic technique and the ability to recognise when a culture has or has not become contaminated are key skills in minimising risk as well as providing reasonable certainty that the intended organism is the one that is being studied. |
| **Culture media** | L1 Organisms can only be cultured on the substances on which they grow naturally, for example, bread, fruit, vegetables, milk, cheese, yoghurt, hay or grass and other plants.<br>L2 Agar-based culture media generally with a simple nutrient base, low pH or high salinity, but not those which select for organisms which are potentially pathogenic to humans, for example, MacConkey's agar. Similar restrictions apply to the equivalent broth media.<br>L3 As for L2, unless very strict precautions are taken to prevent any release of microbes. |
| **Storage of organisms and media** | It is unwise to maintain cultures for long periods, except for some work at L3; they may become contaminated. Every 3 months, organisms should be subcultured and checked for purity (by preparing a streak plate and looking for mixed growth) - but only if aseptic technique can be guaranteed. Whenever mixed growths are found, the stock should be destroyed by autoclaving and a fresh culture obtained. Mixed cultures of protozoa from reputable suppliers can be maintained indefinitely without risk.<br>Cultures, other than those requiring light for their growth and survival, are best stored in the dark at 10-15 °C. If it is impossible to achieve a constant, cool temperature, a refrigerator may be used but never one in which human foodstuffs are kept. Before being used, media should be stored as dry powder or tablets. Prepared media, after sterilisation, can be stored at room temperature for several months in tightly-sealed, screw-topped bottles (preferably medical flats), kept away from direct sunlight. Prepared agar plates can be stored at room temperature; they should be inverted and then kept inside their original plastic sleeves to prevent moisture loss. The plates should be checked before use and any that are contaminated must be discarded. |
| **Disinfectants** | *Virkon* is a broad-spectrum disinfectant. It requires 10 minutes contact time to be effective; an active solution has a bright pink colour but is unlikely to be effective after 36 hours. Do not use old solutions which are pale pink or colourless; they will be inactive. If using a powder, make up a 1% (w/v) solution in water in a fume cupboard to avoid inhaling fine airborne particles. If using tablets, a fume cupboard is unnecessary and 1 tablet makes 500 $cm^3$ in water. Solutions should be made up shortly before the lesson and can be used for disinfecting surfaces and contaminated items.<br>*Biocleanse* is a broad-spectrum disinfectant. It requires 10 minutes contact time to be effective. The concentrate is a blue liquid and the diluted solution is almost colourless; use a 5% (v/v) solution in water. Solutions can be prepared in advance and are active for up to 1 week. The solution can be used for disinfecting surfaces and contaminated items.<br>*Ethanol* (IDA): 70% (v/v) solution in water; it needs 5 minutes contact time to be effective.<br>*Chlorine-based disinfectants* have the disadvantages that they are degraded by organic matter, must be diluted to the correct concentration to match the conditions of use, require 15-30 minutes contact time to be effective, will discolour clothing, and contact with the skin must be avoided. Bleach is therefore not recommended as a general-purpose disinfectant.<br>*Milton* is not suitable as a general-purpose surface disinfectant. *Milton* tablets (not the solution which has a different composition) are used in plant tissue culture to disinfect both plant material and the growth medium. |
| **Bench surfaces** | L2/3 For practical work, bench surfaces should be cleaned beforehand and disinfected as soon as possible afterwards, allowing sufficient time for disinfection to occur. For preparation activities, the work surface must be disinfected before and afterwards |
| **Contamination of technicians, teachers and students** | Before beginning practical work, hands should be washed, preferably with soap and warm water, and must be washed again afterwards. No hand-to-mouth operations, such as chewing, sucking up pipettes or licking labels, should be allowed.<br>L3 All staff and students must wear clean lab coats which can be relatively easily disinfected (as necessary) and then laundered. (Lab coats must always be worn whenever 'self-cloning' microbial transformations are carried out.) |

1. Organisations which can be consulted about the suitability of microorganisms include: the ASE (Association for Science Education)*, CLEAPSS*, MiSAC (Microbiology in Schools Advisory Committee), NCBE (National Centre for Biotechnology Education) and SSERC (Scottish Schools Equipment Research Centre)*. (* Members only.)

| | |
|---|---|
| **Inoculation of cultures** | Media and equipment should be sterile before inoculation. Inoculation should involve precautions to prevent contamination of the person and work surfaces. It should also avoid the contamination of sterile culture media with unwanted microbes. Media must not be deliberately inoculated with sources likely to contain human pathogens.<br>L2/3 For the aseptic transfer of cultures, arrangements should be made to sterilise inoculating loops and glass spreaders before and after inoculation, and to provide discard pots of disinfectant for pipettes and syringes. The mouths of glass culture vessels should be flamed after removing caps and before their replacement. Lids of Petri dishes should be opened only just enough to allow the inoculating tool to be introduced and for as short a time as possible.<br>For further guidance on aseptic technique, refer to Appendix 1 *Subculturing and transfer work at Level 3*. |
| **Incubation** | L1 Incubation should be limited to ambient conditions in the classroom. The only exception will be yoghurt making at 43 °C, which, by using a starter culture and a special medium, is less likely to encourage unwanted, possibly pathogenic, growths.<br>Yeast cultures generate considerable quantities of carbon dioxide gas. Incubation containers should be plugged with cotton wool, or closed with loose-fitting plastic caps, or fermentation locks, which will allow gas to escape.<br>L2/3 The upper limit for general school-based work should be 30° C because, in this temperature range, cultures of microorganisms suitable for school use grow well. In addition, although pathogens can grow on special culture media, there is unlikely to be a hazard when conducting investigations with ordinary culture media and incubation conditions at this temperature using material derived from suitable environments, eg, soil and water. Exceptions to the upper limit of 30º C will include yoghurt making (43° C) and the culturing of *Streptococcus thermophilus* (50° C), *Bacillus stearothermophilus* (60° C) and debilitated strains of *Escherichia coli* (37° C) for work with DNA.<br>Agar plates should be inverted before incubation to avoid condensation dripping onto cultures. During incubation, the lid of the Petri dish should be taped to the base with two or four small pieces of tape so that the lid cannot be accidentally removed and conditions inside cannot become anaerobic. |
| **Spills** | All spills carry a risk of aerosol formation which must be reduced as far as possible. Spills greater than a few drops of any liquid culture should be dealt with using a spills kit by a trained teacher or technician. All spills by students should be reported to the teacher.<br>Students should be moved away from any spill, which is then covered with paper towels soaked in *VirKon* or *Biocleanse* disinfectant. Pour on additional disinfectant if necessary, and leave for at least 10 minutes. Wearing disposable gloves, sweep the spill debris into a dustpan using paper towels and put the debris into a strong plastic bag. Tie up the bag and dispose of it inside another bag which is then sealed. Label the bag 'Broken glass', if appropriate. The dustpan should be left overnight in fresh disinfectant, washed and then reused. Seriously-contaminated clothing should be disinfected before laundering. Contaminated skin should be carefully washed with soap and hot water.<br>Where the spill is only a few drops, wipe up with a paper towel soaked in disinfectant and allow the surface to dry. |
| **Observation of cultures** | L1 Cultures should be viewed in the unopened containers in which they were grown.<br>L2 Cultures should be examined in agar plates which have been taped closed. Unless cultures are known to be of minimum hazard, if there is a risk that students may open them, even though instructed not to do so, it will be prudent for the cultures, after incubation, to be completely sealed with tape around the circumference. If there is a high risk of students deliberately opening such sealed Petri dishes but the teacher wishes to continue the activity, then the cultures must be killed before examination. A filter paper should be placed in the lid of an inverted agar plate, moistened with 40 % methanal solution (formalin) and left for 24 hours. The filter paper is then removed and the dish resealed. (Take care with methanal: the use of eye protection, gloves and a fume cupboard to avoid breathing fumes is essential.)<br>L3 Cultures of known and non-pathogenic microbes can be examined using a variety of techniques. Organisms cultured from body surfaces or any environmental source must be examined in unopened containers, or killed before examination, as above. |
| **Investigations with bioreactors (fermenters)** | L2/3 A bioreactor will require a larger volume of liquid culture medium which poses problems of sterility during preparation and disposal because the volumes of liquid that can be dealt with in school autoclaves / pressure cookers are limited. To make handling easier, choose equipment which keeps quantities to a sensible minimum. To limit contamination, cultures should be started by inoculation with a significant volume of an actively-growing inoculum e.g., 20% of total volume. It is safer to use organisms which require high concentrations of sodium chloride e.g. *Vibrio (Beneckea) natriegens* and *Photobacterium phosphoreum* or produce acid as a consequence of their growth e.g. *Acetobacter aceti* and *Lactobacillus spp*.<br>Guard against the risk of spills of large amounts of liquid culture; for example, place equipment within a tray of sufficient capacity to contain the spill. In the case of gross spills, unless the cultured organism is known to be safe, the laboratory should be cleared before attempting to deal with the spill.<br>L2/3 Many fermentations may generate large volumes of gas, e.g. carbon dioxide or methane. Vessels must be suitably vented to allow the gas to escape without aerosol formation or the entry of contaminant organisms. In the case of methane, the equipment must be kept away from naked flames.<br>The use of animal dung for investigations of biogas generation is not recommended; use grass clippings inoculated with well-rotted garden compost. Other than for work with yeasts and small-scale biogas generation, wholly-anaerobic fermentations should not be studied in schools. Investigations which are partially anaerobic, eg setting up a Winogradsky column, may, however, be attempted. |

| | |
|---|---|
| **Sterilisation and disposal** | Except for Level 1 work using small amounts of material, before disposal, all cultures must be heated to kill microorganisms using a pressure cooker or autoclave. Petri dish cultures and similar waste should be placed inside autoclavable or roasting bags of a suitable size to fit the space inside the equipment. The caps of all screw-topped bottles must be loosened before cultures and media are sterilised.<br>In order to achieve and maintain a sufficiently-high temperature for a long-enough time, it is very important that instructions for the use of an autoclave are followed (but note that those supplied with an older model for sterilising surgical instruments are misleading). Pressure cookers will not be supplied with instructions for sterilisation; refer to Appendix 2 *Steam sterilisation* for further information. Specialist advice and training are available from CLEAPSS and SSERC. Teachers and technicians should be trained to follow safe working practices.<br>Never heat a pressure cooker or autoclave with one or more Bunsen burners; always use a gas ring or electric hotplate.<br>Equipment must be allowed to cool unaided before opening. Rapid cooling and the release of steam are dangerous to the handler and may shatter glassware and/or cause liquid media to boil over. Further information may be sought from CLEAPSS and SSERC.<br>Clean glass equipment can be sterilised by dry heat in an oven (160 °C for at least 2 hours). Wire loops are sterilised by heating to red heat in a Bunsen-burner flame.<br>Sterilisation cannot be achieved by the use of chemical disinfectants or microwave ovens. However, some modern DNA transformation media require microwave heating and include suitable instructions. In addition, microwaves can be used for melting prepared agar media. Ensure any screw tops are loosened beforehand and watch carefully for signs of the medium boiling over. At the first sign of this, pause the microwave oven until the boiling subsides before continuing.<br>After sterilisation, solid cultures can be disposed of, in tied autoclave bags or similar, through the refuse system. Autoclave bags prominently labelled 'Biohazard' should be put in an opaque bag before disposal. Sterilised liquid cultures can be flushed away, preferably down the toilet, or a sink with lots of water. Sterilised culture material should not be allowed to accumulate in waste traps.<br>Incineration is an acceptable alternative to autoclaving. Note, however, that polystyrene Petri dishes will generate hazardous fumes when incinerated; a purpose-built incinerator with a tall flue must be used. |

## Appendix 1 Subculturing and transfer work at Level 3

Work at this level will involve subculturing and transfer work that requires more-sophisticated aseptic techniques. In addition to the safety precautions appropriate for level 2, the following points should be noted.

1 The work area should ideally be on an impervious bench surface such as plastic laminate and away from doors, windows and other direct sources of draughts. Before work is started, the bench should be flooded with a suitable disinfectant, e.g. Ethanol (IDA), *Virkon* or *Biocleanse*.

2 Working close to a Bunsen burner, where the updraught will prevent organisms falling onto apparatus, gives protection to both work and worker.

3 Good microbiological technique is associated with the use of the inoculating loop. Loops can be made by carefully bending 24 s.w.g. nichrome wire round a match stick, to ensure that the loop so formed is fully closed. The overall length of the wire including the loop should be no more than 50 mm. This is to minimise vibration and flicking of material from a charged loop. Loops should be attached to a metal 'chuck'-type holder and not embedded in glass rods. This is because flame sterilisation should include the lower part of the wire where it meets the handle. If a glass rod is used as the handle, it is likely to shatter in the Bunsen-burner flame.

4 A convenient instrument for transferring fungal mycelium can be made by using pliers to bend at a right angle the 3-4 mm tip of a straight inoculation wire. This can then be used to cut and impale pieces of agar from a fungal culture.

5 Any item introduced into a culture must first be sterilised. For an inoculation loop or hook, starting with the part of the wire close to the handle, the entire wire and loop/hook are heated to red heat whilst held almost vertically in the upper part of a blue (roaring) Bunsen-burner flame. Before use, the wire is allowed to cool for about 5 seconds. Direct flaming of a wet loop can cause spluttering; material which spits from the loop may not have been sterilised.

6 Pasteur and graduated pipettes should have their wide ends plugged with non-absorbent cotton wool in an attempt to keep them uncontaminated by dust and microbes. A teat, or a 1 $cm^3$ syringe attached by a short length of silicon tubing, is used to fill the pipette. Plugs are easily penetrated by microorganisms in liquid suspension. If the cotton-wool plug becomes wet, the contaminated pipette should be placed into a discard pot of disinfectant. For some applications, inexpensive micropipettors and autoclavable tips are appropriate. Pasteur and graduated pipettes are sterilised by heating in a hot-air oven, wrapped in either greaseproof paper or aluminium foil and held at 160 °C for 2 hours. A glass spreader is sterilised either as for pipettes or by dipping in and out of a 70% (v/v) ethanol solution (which is then moved away from the Bunsen burner) and igniting the alcohol remaining on the spreader's surface, holding it downwards while aflame.

7 When it is necessary to open culture tubes, Universal bottles, etc, the mouths of the glassware should be warmed by passing them through a blue Bunsen-burner flame. This should be repeated before plugs or caps are replaced. Plugs and caps should not be placed on the bench. With practice, it is possible to manipulate a tube, plug and loop without any of them leaving the hands. Culture tubes and similar glassware should always be supported in a rack, preferably a plastic-coated wire design, to hold them securely.

8 Lids of Petri dishes should be opened just enough to allow the inoculating tool to enter and be manipulated. Lids should be opened for the minimum amount of time necessary for the particular operation to be performed. The lid should be held open at an angle, the opening facing away from the worker.

9 Where 'transfer chambers' are used their limitations should be realised. The chambers on the schools market can help to cut down the general level of contamination of a laboratory by microorganisms from the air and dust. They can also provide a clearly-delineated 'clean' area for post-16 work in laboratories that have to be used by other classes. They are, however, not designed to give protection against potential pathogens and their use does not guard against the consequences of poor technique. In the event of a spill, any aerosol or spore cloud formed may be concentrated right under the nose of the operator.

10 At the end of practical work, the bench should again be disinfected (see 1).

## Appendix 2 Steam sterilisation

Steam sterilisation is the preferred method for both preparation of sterile equipment & media and disposal of agar plates and cultures.

Liquids and equipment are sterilised by steam at 121 °C [103 kPa (kN $m^{-2}$) or 15 lbf $in^{-2}$ steam pressure]. The holding time under these conditions should be at least 15 minutes. In schools, autoclaves are invariably of the non-jacketed, 'pressure-cooker' type. Indeed the 'autoclave' is often a domestic pressure cooker. These vertical, portable laboratory autoclaves are adequate for all normal school work but their limitations should be recognised. Their main disadvantages are that there may be inefficient removal of air before the sterilisation cycle is started and, because of their small size, they are easily overloaded.

Air has an important influence on the efficiency of steam sterilisation. For example, if all the air is removed from the vessel, saturated steam at 103 kPa (kN $m^{-2}$) or 15 lbf $in^{-2}$ has a temperature of 121 °C. With only half the air removed from the autoclave, the temperature of the air-steam mixture is only 112 °C.

In order to arrive at the full 'cycle' time for a vertical autoclave, we must add to the minimum 15-minute holding period at 121 °C:

a) a heating-up period to allow the water to come to the boil;
b) a period of vigorous free steaming to expel air from the equipment and glassware;
c) if the load includes certain 'difficult' materials (see below), an extension of the holding period, possibly but exceptionally as much as an extra 20 minutes;
d) a cooling period (but do not attempt to cool the device rapidly under cold water from a tap or by other means).

The cooling period increases the time of exposure to steam and may be necessary for the effective sterilisation of some materials. In any case, rapid cooling may lead to glassware cracking or liquids boiling over and being wasted. It can be very dangerous to open an autoclave before the pressure has dropped to atmospheric (when the temperature inside will be about 80 °C). The sudden change in temperature caused by opening before the pressure has been allowed to fall has been known to cause violent shattering of glass containers. Serious scalds and burns have occurred because this hazard had not been appreciated.

'Difficult' materials referred to under (c) would not be met frequently in normal school work. Materials such as dry soil will contain heat-resistant spores and will allow steam to penetrate only very slowly. Contaminated cloth can also be difficult because the displacement of trapped air can be a problem.

However, even very exacting samples such as soil caked on tightly-rolled and packed lint have been shown to be reliably sterile after a 35 minute holding time in a domestic pressure cooker. For standard media and recommended 'non-pathogenic' organisms, a 15-20 minute holding period will be effective. Should there be any doubt, the holding time should be increased.