MICROBIOLOGY SOCIETY

Medical Microbiology



About this resource

Microbiology is a popular option for practical work in schools. This manual, which explains the basic techniques necessary to carry out microbiology experiments safely and effectively, is intended as a guide for teachers and technicians.

The Microbiology Society, in association with the Microbiology in Schools Advisory Committee (MiSAC) has also produced a book of 21 practical investigations which complements the manual – *Practical Microbiology for Secondary Schools* is suitable for use with Key Stages 3, 4 and post-16 and the equivalent Scottish qualifications. This resource can be downloaded from the Microbiology Society's dedicated education website: www.microbiologyonline.org.uk

The Microbiology Society offers schools membership and runs an advice service on microbiology teaching. For further details, email education@microbiologysociety.org, telephone +44 (0)20 7685 2682 or write to the address at the bottom of the page.

Other useful sources of advice:

- CLEAPSS – www.cleapss.org.uk
  The Gardiner Building, Brunel Science Park, Uxbridge UB8 3PQ
- Microbiology in Schools Advisory Committee (MiSAC) – www.misac.org.uk
  c/o NCBE *(see address below)*
- National Centre for Biotechnology Education (NCBE) – www.ncbe.reading.ac.uk
  Science and Technology Centre, The University of Reading, 2 Earley Gate, Whiteknights, Reading RG6 6AU
- Scottish Schools Equipment Research Centre (SSERC) – www.sserc.org.uk
  2 Pitreavie Court, South Pitreavie Business Park, Dunfermline KY11 8UB

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Contents

About this resource Inside front cover

Part 1: The Basics
An introduction to microbiology, aseptic technique and safety

Preparation
- Safety guidelines 1
- Risk assessment 2
- Good microbiological laboratory practice (GMLP) 3
- Spillage management 3
- Aerosols 3

Resources
- Equipment 4
- Apparatus 5
- Materials 5

Media, sterilisation and disinfection
- Preparation of culture media 6
- Pouring a plate 6
- Storage of media 6
- Sterilisation vs disinfection 6
- Sterilisation using the autoclave/pressure cooker 7
- Sterilisation of equipment and materials 7
- Choice, preparation and use of disinfectants 7

Inoculation and other aseptic procedures
- Essential points 8
- Using a wire loop 8
- Using a pipette 9
- Flaming the neck of bottles and test tubes 10
- **Working with bacteria and yeast**
  - Streak plate 11
  - Pour plate 12
  - Using a spreader 13
  - Spread plate 14
- **Working with moulds** 15

Incubation 16

In conclusion: clearing up 17

Essential methods for maintaining, preparing and using cultures

Obtaining suitable cultures 18
Pure cultures 18
Maintaining stock cultures 18
Checking cultures for contamination 18
Preventing contamination of cultures and the environment 19
Aseptic transfer of cultures and sterile solutions 19
Preparing cultures for class use 19

Part 2: Microbiology in Action

Practical activities

1. Testing sensitivity to antimicrobial substances 21
2. Microscopy
    - Using the microscope 22
    - Stained preparations 23
    - Making a smear 23
    - A simple stain 24
    - A differential stain: Gram's staining method 24

Appendices

1. Safety guidelines 26
2. Safe micro-organisms 31
3. Safety resources 37
4. Suppliers of cultures and equipment 38
5. Use of the autoclave/pressure cooker 39
6. Preparing serial dilutions 40

Part 1: The Basics

An introduction to microbiology, aseptic technique and safety

As well as causing a familiar range of diseases in animals and plants and problems in food spoilage and deterioration of other materials, microbes are also our 'invisible allies'. Indeed, life on Earth would not be sustainable without the benefits that many of them provide. The teaching of such an important subject as microbiology cannot be achieved effectively without enhancing the theory with 'hands on' experience in the laboratory. The purpose of this manual is to provide teachers and technicians with good techniques in practical microbiology to ensure that investigations proceed safely and achieve the required educational aims successfully. This manual has been written for a right-handed person.

Preparation

Safety guidelines

The small size of microbes and the consequent need to deal with cultures that contain many millions of microbial cells require special procedures for their safe use. Activities involving micro-organisms are controlled by the Control of Substances Hazardous to Health (COSHH) Regulations and teachers and technicians have a duty under the Health and Safety at Work Act to comply with any safety instructions given by their employers. These include using model risk assessments. The publications normally used in secondary schools are:

- *Topics in Safety*, 3rd edition (ASE, 2001)
- *Safeguards in the School Laboratory*, 11th edition (ASE, 2006)
- CLEAPSS Science Publications, CD-ROM (latest edition)
- *Safetynet* (SSERC)
- *Safety in Science Education* (DfEE, available from the ASE website)

The guidelines are straightforward and largely common sense and, as such, are not an obstacle to conducting interesting microbiological investigations in a school laboratory.

Planning ahead is essential when embarking on practical microbiology investigations. There are five areas for consideration.

- Preparation and sterilisation of equipment and culture media.
- Preparation of microbial cultures as stock culture for future investigations and inoculum for the current investigation.
- Inoculation of the media with the prepared culture.
- Incubation of cultures and sampling during growth.
- Sterilisation and safe disposal of all cultures and decontamination of all contaminated equipment.

[Appendix 1: Safety guidelines] [Appendix 3: Safety resources]

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Risk assessment

A full risk assessment must be carried out before embarking on any practical microbiological investigation.

Factors to be considered in risk assessment

Factor	Relevance
Level of practical work [Level 1, Level 2, Level 3, *Topics in Safety*, 3rd edition (ASE, 2001), Topic 15; or *Appendix 1: Safety guidelines*]	Degree of risk of microbial culture; expertise of teacher; age range of students
Choice of micro-organisms (ACDP Hazard Group 1)	Present minimum risk; refer to list of suitable cultures
Source of cultures	Reputable specialist supplier or approved environmental sample
Type of investigations/activities	Adequate containment of cultures; class practical work vs. teacher demonstration
Composition of culture media	Possibility of selecting for growth of pathogens
Volume of cultures	Increased risk with increase in volume of liquid culture
Laboratory facilities	Suitability for level of practical microbiological work
Equipment	Adequate for purpose
Incubation conditions	Possibility of selecting for growth of pathogens
Disposal procedures	Ensures elimination of risk to others
Expertise of technicians and teachers	Competence and level of training in techniques and procedures appropriate to level of practical work
Student age and discipline	Appropriate to level of practical work; confidence in class discipline
Sources of competent advice	ASE*, CLEAPSS*, MISAC, NCBE, SSERC* (**members only*)
Essential reference	See list on p. 1

Key to abbreviations: ACDP, Advisory Committee on Dangerous Pathogens; ASE, Association for Science Education; MISAC, Microbiology in Schools Advisory Committee; NCBE, National Centre for Biotechnology Education; SSERC, Scottish Schools Equipment Research Centre.

[Appendix 1: Safety guidelines]

[Appendix 2: Safe micro-organisms]

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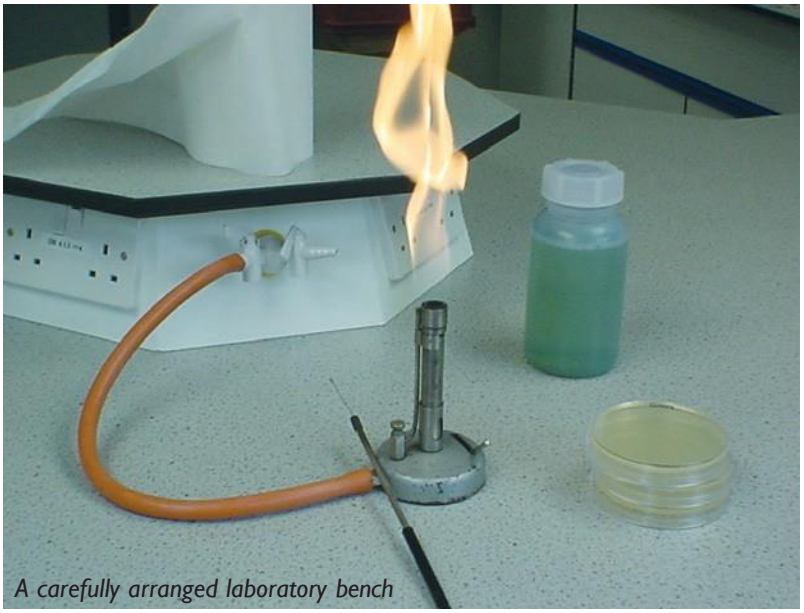
Good microbiological laboratory practice (GMLP)

Training in GMLP is aimed at developing proficiency in containing 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. (*The teacher supervising the practical session must make themselves aware of any medical condition that could cause the student to be at greater risk than average in the laboratory, e.g. treatment with immunosuppressive drugs etc.*)

It is important to arrange the workplace carefully to ensure safe and effective operations.

[Appendix 1: Safety guidelines]



A carefully arranged laboratory bench

Spillage management

Spills

Spillages of cultures must be reported immediately to the teacher or technician to be dealt with quickly. The keeping of a record of all such incidents is recommended. Spilled cultures and surrounding debris (e.g. glass, cotton wool plugs), if any, **must not** be touched with unprotected hands. Wearing disposable gloves, disinfect the area by covering the spill with several layers of paper towel/cloth soaked in a suitable disinfectant (see *Commonly available disinfectants and their uses*, page 7) and leave for 15–30 minutes. Spill debris should then be swept into a dustpan using paper towels. All disposable material should then be transferred to a suitable container, e.g. an autoclave/roasting bag, for autoclaving and disposal. The dustpan must be decontaminated either by autoclaving or by soaking (at least 24 hours) in hypochlorite (sodium chlorate I).

Broken glass

Observe an appropriate disposal procedure for broken glass if present. It should be swept carefully into a suitable container, autoclaved and disposed of in a puncture proof container.

Splashes on clothing and the skin

Contaminated clothing should be soaked in disinfectant. Splashes on the skin should be treated as soon as possible; washing thoroughly with soap and hot water should be sufficient, but if necessary the skin can be disinfected.

Hint

It is useful to have a spillage kit always at hand ready for use.

Suggested components:

- beaker for making fresh disinfectant
- disposable gloves
- dustpan
- paper towel/cloth
- autoclave/roasting bag

Aerosols

Spillages also carry a risk of generating aerosols (an invisible 'mist' of small droplets of moisture) which may contain microbes and might be inhaled. The risk of spillages occurring is lessened by using cultures grown on agar instead of in liquid media whenever possible. Care should also be taken to avoid generating aerosols during practical work. The risk is minimised by adhering to GMLP with special attention to the correct use of pipettes (see *Inoculation and other aseptic procedures* page 8).

Resources

Equipment

Equipment	Use
Loop (wire/plastic)	Routine inoculation of agar slopes/deeps and small volumes of liquid media (up to ca 10 cm^3); making streak plates
Straight wire	Inoculation from very small colonies; transfer of small inocula from liquid media for nutritional work
Spreader (glass/plastic)	Making spread/lawn plates
Forceps (metal/plastic)	Transfer of sterile paper/antibiotic discs; also plant material, e.g. short lengths of root with nodules
Pipette (calibrated/dropping; glass/plastic)	Transfer of measured volumes/drops of culture/sterile solutions (dry, non-absorbent cotton wool plug in neck prevents contamination)
Teat	Filling and emptying pipettes safely (**never** pipette by mouth)
Test tube	Small volumes (ca 5–10 cm^3) of liquid media/agar slopes/sterile solutions for inoculation (held in test tube rack; dry non-absorbent cotton wool plug or plastic cap prevents contamination)
Universal bottle (wide neck); McCartney bottle (narrow neck)	Volumes of liquid and agar media/sterile solutions up to ca 20 cm^3 for inoculation or for storing sterile media or stock cultures on agar slopes (stay upright on bench; plastic screw cap prevents contamination and reduces evaporation during long storage)
Bijou bottle	Very small volumes (up to ca 3 cm^3) of sterile solutions (stay upright on bench; plastic screw cap prevents contamination)
Medical flat	Large volumes of sterile media/solutions for storage; available in range of capacities, 50–500 cm^3 (plastic screw cap prevents contamination and reduces evaporation during long storage)
Conical flask	Large volumes of liquid media for inoculation and liquid/media for short-term storage (non-absorbent cotton wool plug prevents contamination but does not reduce evaporation during long storage)
Petri dish (plastic/glass)	*Plastic*: pre-sterilised for streak/spread/lawn/pour plates; *Glass*: only for materials for sterilisation by hot air oven, e.g. paper discs
Marker pen	Labelling Petri dishes, test tubes, flasks, bottles and microscope slides
Personal protective equipment [Level 2, Level 3, *Topics in Safety*, 3rd edition (ASE, 2001), Topic 15; or *Appendix 1: Safety guidelines*]	*Clean laboratory coat/apron*: protection of clothing, containment of dust on clothing; *Safety spectacles*: not considered essential when dealing with suitable cultures and observing GMLP, but may be required by local regulations and for dealing with chemicals

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Apparatus

Apparatus	Use
Bunsen burner	Sterilisation of wire loops and (with alcohol) metal forceps and glass spreaders
Impervious sheet or tray	Provides individual student working area if the bench surface is not appropriately sealed
Autoclave/pressure cooker	Sterilisation of media, solutions and equipment before use and contaminated items afterwards; melting solidified agar media for use
Gas ring/hot plate	Steam generation in autoclave
Autoclavable/roasting bag	Holds contaminated items in autoclave to contain spillages
Hot air oven	Sterilisation of glass Petri dishes and pipettes and paper discs (but not essential as autoclaves/pressure cookers serve virtually all needs)
Discard pots containing disinfectant	Disposal of used pipettes and slides of non-stained microscopical preparations
Microwave oven	Melting solidified agar media for use (but not in vessels with metal caps and not for sterilisation)
Incubator	Incubation of cultures (but many cultures will grow at room temperature in the interval between lessons)
Water bath	Suitable temperature for keeping melted agar media molten for use (ca 50 °C); accurate temperature control
Thermometer	Checking incubator/water bath temperatures
pH meter	Checking and adjusting pH values of media
Cupboard	Storage of culture media and stock cultures
Refrigerator	Storage of heat-labile materials
Microscope, slides, cover slips, stains, staining rack, immersion oil	Microscopical observations

Materials

Materials	Use
Culture media ingredients	Stock of a range of culture media in dehydrated form (tablets/powder); available as complete media and as separate ingredients
Disinfectants	Treatment of work surface before and after use and spillages; disposal of used pipettes and microscope slides; in soap form for hand washing
Alcohol [70 % industrial denatured alcohol (IDA)]	Sterilisation of metal forceps and glass spreaders by ignition
Autoclave indicator tape	Changes colour in response to heat to distinguish those items that have received heat treatment (but is not an indicator of effective sterilisation)
Steriliser control tube/strip	Changes colour when correct temperature has been applied and held for the required length of time to effect sterilisation
Non-absorbent cotton wool	Plugs for test tubes, flasks and pipettes
Spillage kit	Dealing with spilled cultures

Media, sterilisation and disinfection

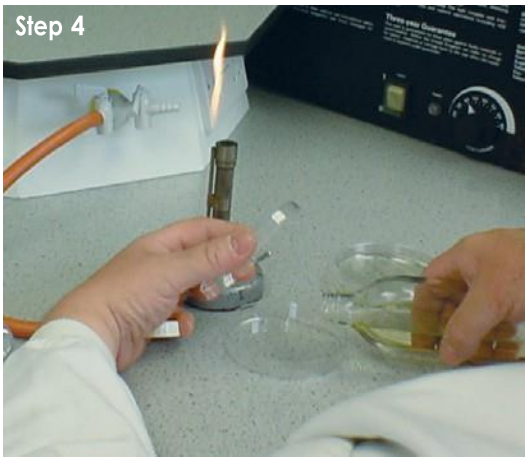
Preparation of culture media

Rehydrate tablets or powder according to manufacturer's instructions. Before sterilisation, ensure ingredients are completely dissolved, using heat if necessary. Avoid wastage by preparing only sufficient for either immediate use (allowing extra for mistakes) or use in the near future. Normally allow 15–20 cm^3 medium per Petri dish. Dispense in volumes appropriate for sterilisation in the autoclave/pressure cooker.

Agar slopes are prepared in test tubes or Universal/McCartney bottles by allowing sterile molten cooled medium to solidify in a sloped position.

Bottles of complete, sterile media are available from suppliers but are expensive.

[Appendix 4: Suppliers of cultures and equipment]



Pouring a plate

1. Collect one bottle of sterile molten agar from the water bath.
2. Hold the bottle in the right hand; remove the cap with the little finger of the left hand.
3. Flame the neck of the bottle.
4. Lift the lid of the Petri dish slightly with the left hand and pour the sterile molten agar into the Petri dish and replace the lid.
5. Flame the neck of the bottle and replace the cap.
6. Gently rotate the dish to ensure that the medium covers the plate evenly.
7. Allow the plate to solidify.

The base of the plate must be covered, agar must not touch the lid of the plate and the surface must be smooth with no bubbles.

The plates should be used as soon as possible after pouring. If they are not going to be used straight away they need to be stored inside sealed plastic bags to prevent the agar from drying out.

Storage of media

Store stocks of prepared media at room temperature away from direct sunlight; a cupboard is ideal but an open shelf is satisfactory. Media in vessels closed by cotton wool plugs/plastic caps that are stored for future use will be subject to evaporation at room temperature; avoid wastage by using screw cap bottles. Re-melt stored agar media in a boiling water bath, pressure cooker or microwave oven. Once melted, agar can be kept molten in a water bath at ca 50 °C until it is ready to be used. Sterile agar plates can be pre-poured and stored in well-sealed plastic bags (media-containing base uppermost to avoid heavy condensation on lid).

Sterilisation vs disinfection

Sterilisation means the complete destruction of all the micro-organisms including spores, from an object or environment. It is usually achieved by heat or filtration but chemicals or radiation can be used.

Disinfection is the destruction, inhibition or removal of microbes that may cause disease or other problems, e.g. spoilage. It is usually achieved by the use of chemicals.

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Sterilisation using the autoclave/pressure cooker

The principle of sterilisation in an autoclave or pressure cooker is that steam under pressure is used to produce a temperature of 121 °C which if held for 15 minutes will kill all micro-organisms, including bacterial endospores.

[Appendix 5: Use of the autoclave/pressure cooker]

Sterilisation of equipment and materials

- Wire loop

  Heat to redness in Bunsen burner flame.

- Empty glassware and glass (not plastic!) pipettes and Petri dishes

  Either: hot air oven, wrapped in either greaseproof paper or aluminium and held at 160 °C for 2 hours, allowing additional time for items to come to temperature (and cool down!).

  Or: autoclave/pressure cooker.

  Note: plastic Petri dishes are supplied in already sterilised packs; packs of sterile plastic pipettes are also available but cost may be a consideration.

- Culture media and solutions

  Autoclave/pressure cooker.

- Glass spreaders and metal forceps

  Flaming in alcohol (70 % IDA).

Choice, preparation and use of disinfectants

Specific disinfectants at specified working strengths are used for specific purposes. The choice is now much more straightforward as the range available from suppliers has decreased.

Commonly available disinfectants and their uses

Disinfectant	Use	Working strength
VirKon	Work surfaces, discard pots for pipettes and slides, skin disinfection	1 % (w/v)
	Spillages	Powder
Hypochlorite (sodium chlorate I)	Discard pots for pipettes and slides	2,500 p.p.m. (0·25 %, v/v) available chlorine
Alcohol	Skin disinfection	70 % (v/v) industrial denatured alcohol (IDA)

When preparing working strength solutions from stock for class use and dealing with powder form, wear eye protection and gloves to avoid irritant or harmful effects.

Disinfectants for use at working strength should be freshly prepared from full strength stock or powder form. Activity of VirKon solution may remain for up to a week (as indicated by retention of pink colour) but less, e.g. 1 day, after use. Use working strength hypochlorite on day of preparation.

Inoculation and other aseptic procedures

Essential points

There are several essential precautions that must be taken during inoculation procedures to control the opportunities for the contamination of cultures, people or the environment.

- Operations must not be started until all requirements are within immediate reach and must be completed as quickly as possible.
- Vessels must be open for the minimum amount of time possible and while they are open all work must be done close to the Bunsen burner flame where air currents are drawn upwards.
- On being opened, the neck of a test tube or bottle must be immediately warmed by flaming so that any air movement is outwards and the vessel held as near as possible to the horizontal.
- During manipulations involving a Petri dish, exposure of the sterile inner surfaces to contamination from the air must be limited to the absolute minimum.
- The parts of sterile pipettes that will be put into cultures or sterile vessels must not be touched or allowed to come in contact with other non-sterile surfaces, e.g. clothing, the surface of the working area, the outside of test tubes/bottles.

Using a wire loop

Wire loops are sterilised using red heat in a Bunsen flame before and after use. They must be heated to red hot to make sure that any contaminating bacterial spores are destroyed. The handle of the wire loop is held close to the top, as you would a pen, at an angle that is almost vertical. This leaves the little finger free to take hold of the cotton wool plug/screw cap of a test tube/bottle.

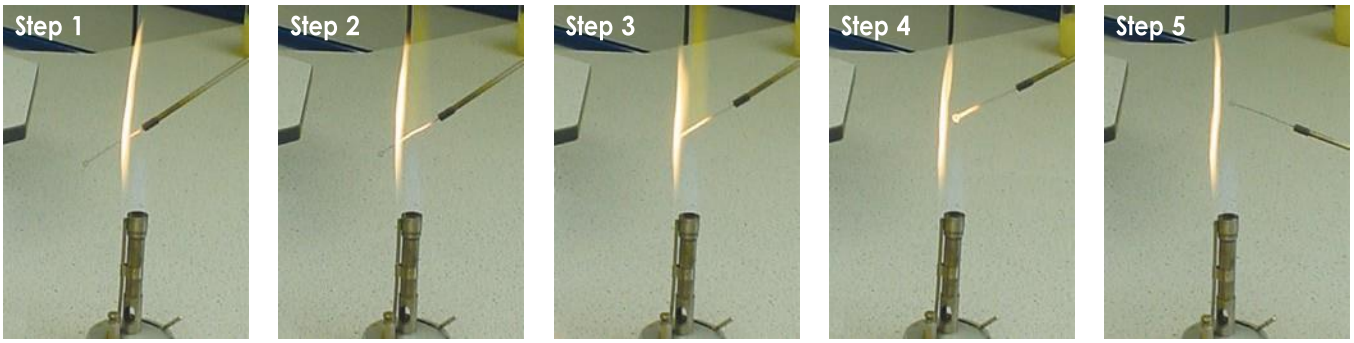
Flaming procedure

The flaming procedure is designed to heat the end of the loop gradually because after use it will contain culture, which may 'splutter' on rapid heating with the possibility of releasing small particles of culture and aerosol formation.

1. Position the handle end of the wire in the light blue cone of the flame. This is the cool area of the flame.
2. Draw the rest of the wire upwards slowly up into the hottest region of the flame, (immediately above the light blue cone).
3. Hold there until it is red hot.
4. Ensure the full length of the wire receives adequate heating.
5. Allow to cool then use immediately.
6. Do not put the loop down or wave it around.
7. Re-sterilise the loop immediately after use.

Hint

If a loop does not hold any liquid the loop has not made a complete circle. To correct the problem, first ensure that the loop has been sterilised and then reshape the loop with forceps. Do not use your fingers because of the possibility of puncturing the skin.



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Using a pipette

Sterile graduated or dropping (Pasteur) pipettes are used to transfer cultures, sterile media and sterile solutions.

1. Remove the pipette from its container/ wrapper by the end that contains a cotton wool plug, taking care to touch no more than the amount necessary to take a firm hold.
2. Fit the teat.
3. Hold the pipette barrel as you would a pen but do not grasp the teat.

   The little finger is left free to take hold of the cotton wool plug/cap of a test tube/ bottle and the thumb to control the teat.
4. Depress the teat cautiously and take up an amount of fluid that is adequate for the amount required but does not reach and wet the cotton wool plug.
5. Return any excess gently if a measured volume is required.

   The pipette tip must remain beneath the liquid surface while taking up liquid to avoid the introduction of air bubbles which may cause 'spitting' and, consequently, aerosol formation when liquid is expelled.
6. Immediately after use put the now contaminated pipette into a nearby discard pot of disinfectant.

   The teat must not be removed until the pipette is within the discard pot otherwise drops of culture will contaminate the working surface.













Hints

- A leaking pipette is caused by either a faulty or ill-fitting teat or fibres from the cotton wool plug between the teat and pipette.
- A dropping (Pasteur) pipette can be converted to delivering measured volumes by attaching it to a non-sterile syringe barrel by rubber tubing.
- Commercial dispensing systems are available such as measuring Pasteur pipettes.

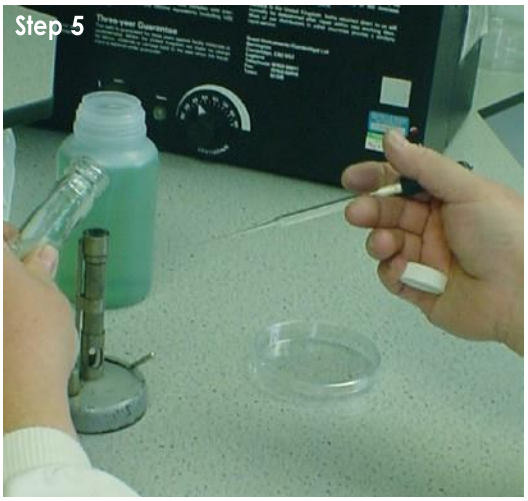


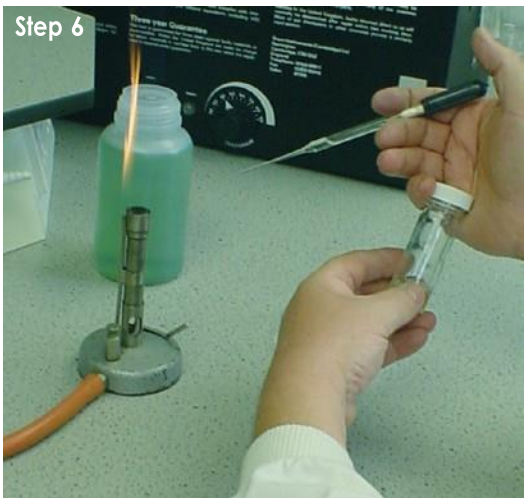
Converting a Pasteur pipette by attaching a syringe barrel

[Appendix 4: Suppliers of cultures and equipment]

Flaming the neck of bottles and test tubes

1. Loosen the cap of the bottle so that it can be removed easily.
2. Lift the bottle/test tube with the left hand.
3. Remove the cap of the bottle/cotton wool plug with the little finger of the right hand. (Turn the bottle, not the cap.)
4. Do not put down the cap/cotton wool plug.
5. Flame the neck of the bottle/test tube by passing the neck forwards and back through a hot Bunsen flame.
6. After carrying out the procedure required, e.g. withdrawing culture, replace the cap on the bottle/cotton wool plug using the little finger. (Turn the bottle, not the cap.)





Hints

- Label tubes and bottles in a position that will not rub off during handling. Either marker pens or self-adhesive labels are suitable.
- Occasionally cotton wool plugs accidentally catch fire. Douse the flames by immediately covering with a dry cloth, not by blowing or soaking in water.

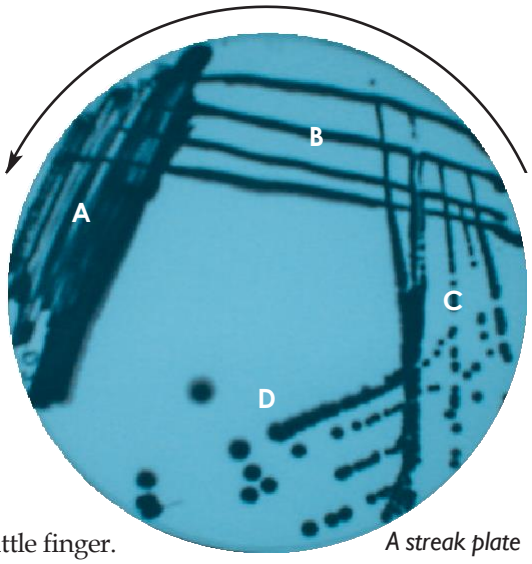
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Working with bacteria and yeast

Streak plate

The loop is used for preparing a streak plate. This involves the progressive dilution of an inoculum of bacteria or yeast over the surface of solidified agar medium in a Petri dish in such a way that colonies grow well separated from each other.

The aim of the procedure is to obtain single isolated pure colonies.



A streak plate

1. Loosen the cap of the bottle containing the inoculum.
2. Hold the loop in the right hand.
3. Flame the loop and allow to cool.
4. Lift the bottle/test tube containing the inoculum with the left hand.
5. Remove the cap/cotton wool plug of the bottle/test tube with the little finger of the right hand.
6. Flame the neck of the bottle/test tube.
7. Insert the loop into the culture broth and withdraw.

At all times, hold the loop as still as possible.

8. Flame neck of the bottle/test tube.
9. Replace the cap/cotton wool plug on the bottle/test tube using the little finger. Place bottle/test tube on bench.
10. Partially lift the lid of the Petri dish containing the solid medium.
11. Hold the charged loop parallel with the surface of the agar; smear the inoculum backwards and forwards across a small area of the medium (see streaked area 'A' in photograph).
12. Remove the loop and close the Petri dish.
13. Flame the loop and allow it to cool. Turn the dish through 90° anticlockwise.
14. With the cooled loop streak the plate from area 'A' across the surface of the agar in three or four parallel lines ('B'). Make sure that a small amount of culture is carried over.
15. Remove the loop and close the Petri dish.
16. Flame the loop and allow to cool. Turn the dish through 90° anticlockwise again and streak from 'B' across the surface of the agar in three or four parallel lines ('C').
17. Remove the loop and close the Petri dish.
18. Flame the loop and allow to cool. Turn the dish through 90° anticlockwise and streak loop across the surface of the agar from 'C' into the centre of the plate ('D').
19. Remove the loop and close the Petri dish. Flame the loop.
20. Seal and incubate the plate in an inverted position.

There are alternative methods for preparing a streak plate but the method shown is the most straightforward.

Hints

- Label the half of the dish that contains medium; use abbreviations and keep them to the edge of the plate so as not to interfere with the later observation of colonies. The same applies to the pour and spread plates described below. Either marker pens or self-adhesive labels are suitable.
- There are two approaches to making a streak plate: (1) with the base (containing medium) placed on the working surface, lift the lid vertically (i.e. still covering the base) the least amount that will allow access of the loop; (2) with the lid placed on the working surface, lift out the base, invert it and inocu late the upwards- facing agar surface. The second method is best reserved for older students working in a relatively dust and draught-free laboratory; it is the one used by professional microbiologists.

Pour plate

A pour plate is one in which a small amount of inoculum from broth culture is added by pipette to the centre of a Petri dish. Molten, cooled agar medium in a test tube or bottle, is then poured into the Petri dish containing the inoculum. The dish is gently rotated to ensure that the culture and medium are thoroughly mixed and the medium covers the plate evenly. Pour plates allow micro-organisms to grow both on the surface and within the medium. Most of the colonies grow within the medium and are small in size and may be confluent; the few that grow on the surface are of the same size and appearance as those on a streak plate.

If the dilution and volume of the inoculum, usually 1 cm^3, are known, the viable count of the sample, i.e. the number of bacteria or clumps of bacteria, per cm^3 can be determined. The dilutions chosen must be appropriate to produce between 30 and 100 separate countable colonies. *[Appendix 6: Preparing serial dilutions]*

Inoculation using a Pasteur pipette

1. Loosen the cap/cotton wool plug of the bottle containing the inoculum.
2. Remove the sterile Pasteur pipette from its container, attach the bulb and hold in the right hand.
3. Lift the bottle/test tube containing the inoculum with the left hand.
4. Remove the cap/cotton wool plug with the little finger of the right hand.
5. Flame the bottle/test tube neck.
6. Squeeze the teat bulb of the pipette very slightly, put the pipette into the bottle/test tube and draw up a little of the culture. Do not squeeze the teat bulb of the pipette after it is in the broth as this could cause bubbles and possibly aerosols.
7. Remove the pipette and flame the neck of the bottle/test tube again, before replacing the cap/cotton wool plug.
8. Place bottle/test tube on bench.

At all times hold the pipette as still as possible.

Inoculating the Petri dish

1. Lift the lid of the Petri dish slightly with the right hand and insert the pipette into the Petri dish and gently release the required volume of inoculum onto the centre of the dish. Replace the lid.
2. Put the pipette into a discard pot. Remove the teat while the pipette is pointing into the disinfectant.



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Pouring the plate

1. Collect one bottle of sterile molten agar from the water bath.
2. Hold the bottle in the right hand; remove the cap with the little finger of the left hand.
3. Flame the neck of the bottle.
4. Lift the lid of the Petri dish slightly with the left hand and pour the sterile molten agar into the Petri dish and replace the lid.
5. Flame the neck of the bottle and replace the cap.
6. Gently rotate the dish to mix the culture and the medium thoroughly and to ensure that the medium covers the plate evenly.
7. Allow the plate to solidify.
8. Seal and incubate the plate in an inverted position.

The base of the plate must be covered, agar must not touch the lid of the plate and the surface must be smooth with no bubbles.







Hints

- Use a water bath at 50 °C to store bottles of molten agar.
- Ensure that the temperature of the molten agar is cool enough for mixing with the culture.
- Take care not to contaminate the molten agar in the bottles with water from the water bath.
- To avoid contamination ensure:
  - that the water in the water bath is at the right depth
  - the bottles are kept an upright position
  - that the outsides of the bottles are wiped before they are used

Using a spreader

Sterile spreaders are used to distribute inoculum over the surface of already prepared agar plates.

Hint

It is advisable to use agar plates that have a well-dried surface so that the inoculum dries quickly. Dry the surface of agar plates by either incubating the plates for several hours, e.g. overnight, beforehand or put them in a hot air oven (ca 55–60 °C) for 30–60 minutes with the two halves separated and the inner surfaces directed downwards.

Wrapped glass spreaders may be sterilised in a hot air oven (see *Media, sterilisation and disinfection* page 6). They can also be sterilised by flaming with alcohol.

Sterilisation using alcohol

1. Dip the lower end of the spreader into a small volume of alcohol (70 % IDA) contained in a vessel with a lid (either a screw cap or aluminium foil).
2. Pass quickly through a Bunsen burner flame to ignite the alcohol; the alcohol will burn and sterilise the glass.
3. Remove the spreader from the flame and allow the alcohol to burn off.
4. Do not put the spreader down on the bench.

Hints

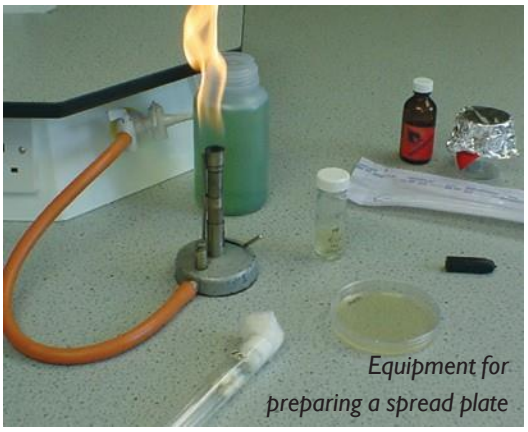
- Ensure that the spreader is pointing downwards when and after igniting the alcohol to avoid burning yourself.
- Keep the alcohol beaker covered and away from the Bunsen flame.

Spread plate

Spread plates, also known as lawn plates, should result in a heavy, often confluent growth of culture spread evenly over the surface of the growth medium. This means that they can be used to test the sensitivity of bacteria to many antimicrobial substances, for example mouthwashes, garlic, disinfectants and antibiotics.

The spread plate can be used for quantitative work (colony counts). If the dilution and volume of the inoculum, usually 0·1 cm^3, are known, the viable count of the sample, i.e. the number of bacteria or clumps of bacteria per cm^3, can be determined. The dilutions chosen must be appropriate to produce between 30 and 100 separate countable colonies.

[Appendix 6: Preparing serial dilutions]



Equipment for preparing a spread plate









1. Loosen the cap of the bottle/test tube containing the broth culture.
2. Remove a sterile Pasteur pipette from its container and attach the bulb held in the right hand.
3. Hold a sterile pipette in the right hand and the bottle/test tube containing the broth culture in the left.
4. Remove the cap/cotton wool plug of the bottle/test tube with the little finger of the right hand and flame the neck.
5. With the pipette, remove a small amount of broth.
6. Flame the neck of the bottle/test tube and replace the cap/plug.
7. With the left hand, partially lift the lid of the Petri dish containing the solid nutrient medium.
8. Place a few drops of culture onto the surface about 0·1 cm^3 (ca 5 drops, enough to cover a 5 pence piece).

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9. Replace the lid of the Petri dish.
10. Place the pipette in a discard jar.
11. Dip a glass spreader into alcohol (70 % IDA), flame and allow the alcohol to burn off.
12. Lift the lid of the Petri dish to allow entry of spreader.
13. Place the spreader on the surface of the inoculated agar and move the spreader in a top-to-bottom or a side-to-side motion to spread the inoculum over the surface of the agar. Make sure the entire agar surface is covered.

This operation must be carried out quickly to minimise the risk of contamination.

14. Replace the lid of the Petri dish.
15. Flame spreader using alcohol.
16. Let the inoculum dry.
17. Seal and incubate the plate in the inverted position.









Hint

The calibrated drop (Miles u Misra) method for colony counts of pure cultures of bacteria and yeast is a more economical method than pour and spread plates. The procedure is as for the spread plate but fewer plates are needed because: (1) the inoculum is delivered as drops from a dropping pipette that is calibrated (by external diameter of the tip) to deliver drops of measured volume e.g. 0·02 cm^3; (2) many drops (six or more) can be put on one plate. The method is not usually suitable for mixed cultures obtained from natural samples, e.g. soil.

Working with moulds

It is sometimes appropriate to prepare a mould inoculum as a spore suspension (particular care is necessary to prevent them from escaping into the air), but often the inoculum is a portion of the mycelium taken with a loop or straight wire with the end few millimetres bent at a right angle. When an agar plate with a mould inoculated at the centre is required, it is easy to inoculate accidentally other parts of the plate with tiny pieces of mould, usually spores, that fall off the loop or wire. This can be avoided by placing the Petri dish on the working surface lid down, lifting the base (containing medium) vertically above the lid and introducing the inoculum upwards onto the centre of the downwards-facing agar surface with a bent wire.

Incubation

Note the previous comments on labelling (see *Inoculation and other aseptic procedures* page 11).

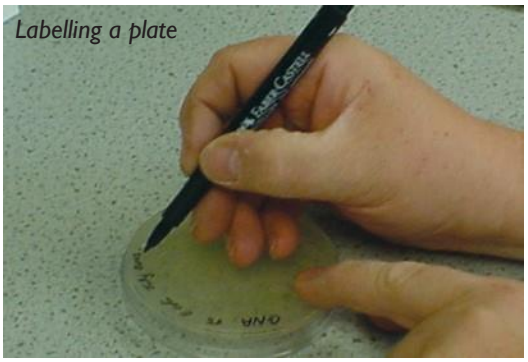
For guidance on incubation temperatures see *Appendix 1: Safety guidelines.*

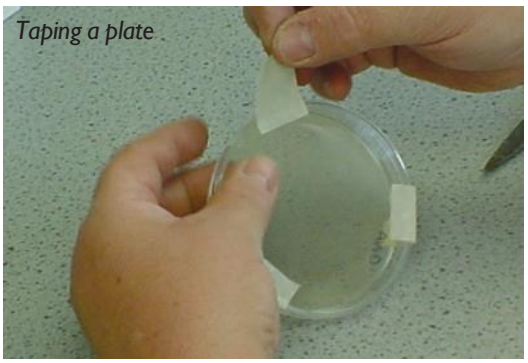
The lid and base of an agar plate should be taped together with 2–4 short strips of adhesive tape as a protection from accidental (or unauthorised!) opening during incubation.

Agar plates must be incubated 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 risk the spillage of the contaminated liquid.

The advantages of incubators are that they may be set at a range of temperatures and reduce the possibility of cultures being interfered with or accidentally discarded. However, many cultures suitable for use in schools will grow at room temperature in the interval between lessons and can be incubated satisfactorily in a cupboard. The temperature of an incubator varies from the set temperature, oscillating by several degrees in the course of use.

Water baths are used when accurately controlled temperatures are required, e.g. for enzyme reactions and growth-temperature relationships, when temperature control of incubators is not sufficiently precise. They should be used with distilled or deionised water to prevent corrosion and emptied and dried for storage.


Labelling a plate


Taping a plate

Hint

Overlong incubation of mould cultures will result in massive formation of spores which readily escape, particularly from Petri dishes, and may cause contamination problems in the laboratory and be a health hazard. This can occur in an incubator, at room temperature and even in a refrigerator.

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In conclusion: clearing up

Working surfaces must be cleared after use. If cultures have been used the benches must be swabbed with disinfectant (VirKon; see *Choice, preparation and use of disinfectants* page 7).

Discarded cultures, empty media tubes and all contaminated material must be placed in the appropriate labelled receptacles. Discard containers must be carefully and securely packed and never overloaded. Plastic Petri dishes must never be stacked above the lip of the discard container.

Cultures and contaminated paper towels, gloves, etc., must be autoclaved at 121 °C for 15 minutes before disposal.

Slides, pipettes and Pasteur pipettes must be discarded in the appropriate containers of hypochlorite (sodium chlorate 1) (see *Choice, preparation and use of disinfectants* page 7). They must be soaked for at least 24 hours before disposal.

Never discard sharp or broken items in a way which would endanger anyone (see *Spillage management* page 3).

After sterilisation, all materials can be disposed of with normal waste. Care must be taken that glass is adequately packaged to prevent injury.

Before leaving the laboratory, laboratory coats must be removed and hands washed thoroughly with hot water and soap.



Pouring disinfectant on the bench



Swabbing with disinfectant



Washing hands

Essential methods for maintaining, preparing and using cultures

Obtaining suitable cultures

Micro-organisms on the list approved for use in schools and colleges (see *Preparation* page 2) present minimum risk given observance of GMLP. The list is not definitive; other organisms may be used if competent advice is taken. Ensure that the current version of the list is consulted because recommendations are altered from time to time with changes in experience and assessment of the risks. Cultures must be obtained from a reputable specialist schools supplier. Isolation of cultures from the environment may be conducted if appropriate to the level of work (i.e. Level 1, Level 2 or Level 3; see *Appendix 1: Safety guidelines*). *[Appendix 2: Safe micro-organisms; Appendix 4: Suppliers of cultures and equipment]*

Pure cultures

The ability to keep pure cultures from becoming contaminated during inoculation and use is a key feature of GMLP. This skill is crucial for reasons of safety and for maintaining the scientific integrity of an investigation. Clearly, it is also a vital skill to recognise when a culture has become contaminated.

Maintaining stock cultures

It may be convenient to maintain a stock of a pure culture instead of re-purchasing it when needed. Most of those considered suitable for use are also relatively easy to maintain by sub-culturing on the medium appropriate for growth but maintenance of stock cultures needs to be well organised with attention to detail. Be prepared to transfer cultures four times a year to maintain viability. Cultures on streak plates are not suitable as stock cultures. Slope cultures in screw cap bottles are preferred because the screw cap reduces evaporation and drying out and cannot be accidentally knocked off (cf. a streak plate culture). Slope cultures are preferred to broth (i.e. liquid medium) cultures because the first sign of contamination is much more readily noticed on an agar surface.

Two stock cultures should be prepared; one is the 'working' stock for taking sub-cultures for classes, the other is the 'permanent' stock which is opened only once for preparing the next two stock cultures. Incubate at an appropriate temperature until there is good growth.

For growing strict aerobes it may be necessary to slightly loosen the cap for incubation (but close securely before storage) if there is insufficient air in the headspace.

As soon as there is adequate growth, the cultures may be stored in a refrigerator, but never one in which human foodstuffs are kept. However, they will remain viable at room temperature in either a cupboard or drawer.

Keep on the lookout for contamination.

Checking cultures for contamination

Evidence for a culture being pure or otherwise is given by the appearance of colonies on a streak plates and of cells in a stained microscopical preparation. There should be uniformity of colony form and cell form (and consistency with the appearance of the original culture!). It is sensible to check purity on suspicion of contamination of the working stock culture from time to time and of the permanent stock when preparing new stock cultures.

If a culture becomes contaminated, it is not advisable to try to remedy the situation by taking an inoculum from a single colony from a streak plate of the mixed culture because of the possibility of (1) not being able to distinguish between the colony forms of the contaminant and the original culture, and (2) culturing a variant of the original culture that does not behave as the original culture did. Instead, go back to the working (or permanent) stock cultures; that's what they are for!

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Preventing contamination of cultures and the environment

Cotton wool plugs

Plugs made of **non-absorbent** cotton wool are used in test tubes and pipettes to prevent micro-organisms from passing in or out and contaminating either the culture or the environment. The necessary movements of air in and gaseous products out are not prevented and the gaps between the cotton wool fibres are even wide enough for micro-organisms to pass through. However, this does not happen because micro-organisms (negatively charged) are 'filtered' out by being attracted to and adsorbed on the oppositely charged cotton wool. The cotton wool must remain dry because this filtration property is lost if the cotton wool becomes moist – hence the use of **non-absorbent** cotton wool.

For use in test tubes a plug should be properly made to ensure that it can be held comfortably without being dropped and its shape and form are retained while being removed from and returned to a test tube several times. Aseptic technique cannot be maintained with poorly made plugs; working surfaces, floors and cultures may become contaminated and students may become understandably (but avoidably) frustrated and lose interest.

Aseptic transfer of cultures and sterile solutions

Regular practice is necessary to ensure that the manipulations involved in aseptic transfer of cultures and sterile solutions become second nature.

Making a **streak plate** is a basic procedure that tests several skills and serves several purposes. During the inoculation procedure, the agar surface is protected from contamination by micro-organisms that are carried in the air by keeping the time that the Petri dish is open to a minimum (see *Streak plate*, page 11).

The choice of **loop or pipette** for transfers between test tubes and screw cap bottles depends on whether they contain agar slopes, liquid media or sterile solutions.

The wire loop is usually satisfactory for inoculating a tube or bottle from a separate colony on a plate but a straight wire is occasionally needed for dealing with very small colonies such as occur with pure cultures of some bacteria, e.g. species of *Streptococcus* and *Lactobacillus*, and on plates that are being used for isolating cultures from natural samples.

Preparing cultures for class use

Microbial cultures cannot be taken from a shelf and instantly be ready for use. It is necessary to begin to prepare cultures well in advance otherwise the outcome might not be as expected. The key is to transfer cultures several times in advance to ensure that they are growing well and are presented as young, fully active cultures on the day of the practical class. For most cultures of **bacteria and yeasts** this will be after incubation for 1 or 2 days; progress of growth can be followed by observation with the naked eye, looking for growth on an agar surface or turbidity in a broth culture. It is usual to grow **moulds** on the surface of an agar medium, allowing an incubation period of from several days to a week.

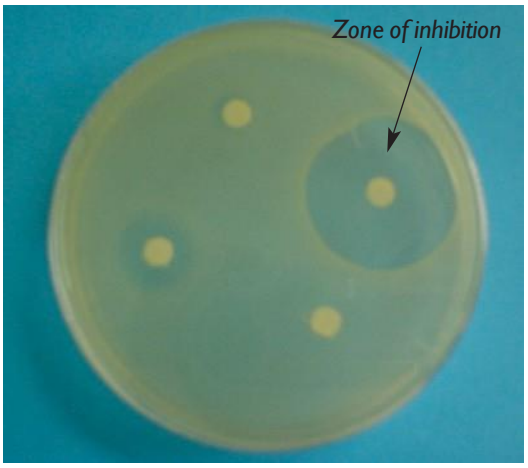
The main points to observe are use of an adequate amount of inoculum, an appropriate culture medium and incubation temperature and, if it is necessary to grow a strictly aerobic organism in a single large volume of liquid culture (i.e. more than ca 20 cm^3), provision of adequate aeration.

It will save time in preparing large numbers of cultures of bacteria and yeast for the class if the inoculum is taken by Pasteur pipette from a well-growing (i.e. turbid) broth culture. A line of growth on a slope culture inoculated by wire loop is easy for students to observe but almost the same effect can be achieved with a pipette.

Part 2: Microbiology in action

Practical activities

1. Testing sensitivity to antimicrobial substances



The agar diffusion method is widely used in industry for testing the sensitivity of micro-organisms to antibiotics, antiseptics, toothpaste, mouthwashes, disinfectants, etc. The method involves preparing a pour or spread plate of a test micro-organism, adding small amount of test substance to either a well cut in the agar medium or (preferably) a paper disc which is then placed on the agar surface. After incubation, an inhibitory effect on the test organism is indicated by a clear zone (no growth) around the test substance; microbial growth is visible to the naked eye in areas of the plate that are unaffected.

This is a straightforward activity that tests several practical skills and is relevant to other aspects of biology and to everyday life. In addition to using laboratory reagents, e.g. stains, and antibiotic discs, many preparations with antimicrobial activity are readily available in pharmacists and supermarkets. There is also the opportunity to think of less obvious materials, e.g. plants and their products.

Materials

- Take a nutrient agar pour or spread/lawn plate of e.g. *Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli* or *Saccharomyces cerevisiae* on malt agar.
- Sterile filter paper discs
- Sterile distilled/demineralised water (control)
- Samples to be tested, 3 (e.g. mouthwashes, selected for a range of active ingredients)
- Bunsen burner
- Forceps
- Alcohol (70 % IDA) in a small beaker covered in foil (**Caution**: flammable, should be kept covered away from flames)
- Incubator at 25–30 °C (if available)

Procedure

Aseptic technique should be used throughout.

1. Mark and label four sections on the base of the Petri dish, for the three different samples and control (sterile water).
2. Using sterile forceps (flamed with alcohol and cooled) remove one filter paper disc. Dip into the first test sample, drain on the side of the container and place firmly onto the appropriate section of the seeded agar plate.
3. Wash the forceps free of the sample.
4. Repeat for the remaining samples and the control (sterile water). Remember to rinse and sterilise the forceps between each sample and to open the plate for the minimum possible time.
5. Seal the lid to the base with tape.

Incubation of the plate.

6. Invert the plate and incubate at 25–30 °C or at room temperature for 48 hours.
7. Examine the plate (without opening). Measure and record the size of any zones of inhibition around the filter paper discs. Consider what factors might be affecting the size of the zones of inhibition.

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2. Microscopy

Using the microscope

The setting up of a microscope is a basic skill of microbiology yet it is rarely mastered. Only when it is done properly can the smaller end of the diversity of life be fully appreciated and its many uses in practical microbiology, from aiding identification to checking for contamination, be successfully accomplished. The amount of magnification of which a microscope is capable is an important feature but it is the resolving power that determines the amount of detail that can be seen.

[Appendix 3: Safety resources]

Hints

- Adjust the iris diaphragm to achieve optimum balance between definition and glare. Do not control light intensity by moving the sub-stage condenser, the position of which should be to focus the light on the specimen. Re-adjust the iris diaphragm for each objective lens.
- For looking at wet mounts of living specimens of protozoa, algae, moulds and even yeasts, the low power objective lens (×10) is often adequate but also necessary for locating and centering on an area of interest before turning to the high power objective lens (×40). Without altering the focus, turn to the high power lens and then finely re-focus.
- Use the oil immersion objective lens for examining stained preparations of bacteria. Put one drop of immersion oil onto the preparation; a coverslip is not required. Remove the slide and wipe the oil immersion lens clean at the end of the practical session.

Bacteria and yeast

Yeast can be seen in unstained wet mounts at magnifications ×100. Bacteria are much smaller and can be seen unstained at ×400 but only if the microscope is properly set up and all that is of interest is whether or not they are motile. A magnification of ×1,000 and the use of an oil immersion objective lens for observing stained preparations are necessary for seeing their characteristic shapes and arrangements. The information gained, along with descriptions of colonies, is the starting point for identification of genera and species, but further work involving physiology, biochemistry and molecular biology is then needed.

Moulds

Routine identification of moulds is based entirely on the appearance of colonies to the naked eye and of the mycelium and spores in microscopical preparations.

Mould mycelium and spores can be observed in unstained wet mounts at magnifications of ×100 although direct observations of 'mouldy' material through the lid of a Petri dish or specimen jar at lower magnifications with the plate microscope are also informative (but keep the lid on!). Routine identification of moulds is based entirely on the appearance of colonies to the naked eye and of the mycelium and spores in microscopical preparations.

Hint

Preparing a quick temporary mount for looking at living protozoa or algae.

Take a microscope slide and place 1 or 2 loopfuls of the sample in the centre. Place a coverslip over the sample, avoiding air bubbles. Seal each edge of the coverslip in turn with a thin film of Vaseline from the warmed end of a microscope slide. Keep microscope lamps as dim as possible to stop the culture from drying out.

Protozoa and algae

Protozoa and algae are large organisms and therefore are readily visible at a magnification of ×10 to ×100 in unstained wet mounts. A magnification of ×100 is advantageous for observing natural samples that contain a variety of organisms, particularly as many are very motile. Identification of algae and protozoa is based entirely on their microscopical appearance. The common algae are green and non-motile; diatoms have a brown, sculptured outer layer of silica and move slowly. Protozoa are colourless and most are motile. Hay infusions and cloudy vase water are rich in algae and protozoa, but clear samples of water are rarely rewarding.

For further information contact Sciento and see *Appendix 4: Suppliers of cultures and equipment.*

Stained preparations

A 'smear' of bacteria or yeast is made on a microscope slide, fixed, stained, dried and, without using a coverslip, examined with the aid of a microsope. Aseptic technique must be observed when taking samples of a culture for making a smear. A culture on agar medium is much preferable to a liquid culture for making a smear. A smear that is thin and even enables the shape and arrangement of cells to be clearly seen and ensures that the staining procedure is applied uniformly. There are two broad types of staining method:

(1) a **simple stain** involves the application of one stain to show cell shape and arrangement and, sometimes, inclusions that do not stain, e.g. bacterial endospores;

(2) a **differential stain** involves a sequence of several stains, sometimes with heating, and includes a stage which differentiates between either different parts of a cell, e.g. areas of fat storage, or different groups, e.g. between Gram-positive and Gram-negative bacteria. The reaction of bacteria to Gram's staining method is a consequence of differences in the chemical structure of the bacterial cell wall and is a key feature in their identification. Yeast cells can be stained by Gram's method, but it is of no value in their identification.

The basis of Gram's staining method is the ability or otherwise of a cell stained with crystal violet to retain the colour when treated with a differentiating agent, usually alcohol (although professionals sometimes use acetone). Bacteria that retain the violet/purple colour are called Gram-positive. Those that lose the colour, i.e. Gram-negative, are stained in the contrasting colour of a counterstain, usually pink/red.

Making a smear

1. Clean a plain microscope slide thoroughly using lens tissue.
2. Label a microscope slide with a marker pen to record the culture being used, date and initials; this is also a useful reminder of which side of the slide is being used.
3. Flame a wire loop to ensure that no culture accidentally remains from a previous operation.
4. Transfer one or two loopfuls of tap water on to the centre of the slide.
5. Flame loop and allow to cool.
6. Using aseptic technique, transfer a very small part of a single colony from a plate or slope of agar medium into the tap water.

If the amount of culture on the loop is easily visible you have taken too much!

7. Make a suspension of the culture in the tap water on the slide and thoroughly but gently spread it evenly over an oval area of up to 2 cm length.
8. Flame the loop.

If it is necessary to use a liquid culture or sample, the use of tap water to prepare the smear will probably be unnecessary and may result in a smear with too few cells.

9. Dry the suspension by warming gently over a Bunsen burner flame and then 'fix' it by quickly passing it through the flame a few times.

This is called a **heat-fixed smear**; it should be visible to the naked eye as a whitish area. Fixing is necessary to ensure that cells adhere to the slide and to minimise any post-mortem changes before staining.

The smear is now ready to be stained.

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A simple stain

1. Put the slide with the fixed smear uppermost on a staining rack over a sink or staining tray.
2. Thoroughly cover the smear with stain and leave for, usually, 30 seconds.
3. Hold the slide with forceps (optional but avoids stained fingers), at a 45° angle over the sink.
4. Rinse off the stain with tap water.
5. Blot-dry the smear with filter/fibre-free blotting paper using firm pressure, but not sideways movements that might remove the smear.
6. Examine under oil immersion.
7. When finished, dispose of slides into discard jar.

Suitable stains include basic dyes (i.e. salts with the colour-bearing ion, the chromophore, being the cation), such as methylene blue, crystal violet and safranin.

A differential stain: Gram's staining method

Times of the staining periods depend on the formulation of the staining solutions which are not standard in all laboratories. Therefore, the times given relate only to the solutions specified here.

1. Put the slide with the fixed smear uppermost on a staining rack over a sink or staining tray.
2. Thoroughly cover the smear with crystal violet solution and leave for 1 minute.
3. Hold the slide with forceps (optional but avoids stained fingers), at a 45° angle over the sink.
4. Pour off the stain, wash off any that remains (and any on the back of the slide) with iodine solution.
5. Put the slide back on the staining rack.
6. Cover the smear with iodine solution and leave for 1 minute. Iodine solution acts as a 'mordant' (a component of a staining procedure that helps the stain to adhere to the specimen), a crystal violet–iodine complex is formed and the smear looks black.
7. Hold the slide with forceps at a 45° angle over the sink and wash off the iodine solution with 70% IDA (not water); continue treating with alcohol until the washings are pale violet.
8. Rinse immediately with tap water.
9. Put the slide back on the staining rack.
10. Cover the smear with the counterstain, e.g. aqueous safranin solution, 0·5 % (w/v), for 30 seconds.
11. Rinse off the stain with tap water.
12. Blot dry the smear with filter/fibre free blotting paper using firm pressure but not sideways movements that might remove the smear.
13. Examine under oil immersion.
14. When finished, dispose of slides into discard jar.

Gram-positive bacteria appear – **VIOLET/PURPLE**

Gram-negative bacteria appear – **RED/PINK**

Examples of typical Gram stain results are as follows.

Gram-positive bacteria	Gram-negative bacteria
Bacillus megaterium	*Escherichia coli*
Bacillus subtilis	*Pseudomonas fluorescens*
Lactobacillus spp.	
Micrococcus luteus	
Staphylococcus albus	
Streptococcus lactis	

Yeasts appear violet and red, but this has no taxonomic significance.

Reagents

Alcohol:

70% IDA

Crystal violet solution:

A	Crystal violet	2·0 g
	Absolute alcohol	100 ml
B	Ammonium oxalate	1·0 g
	Distilled/deionised water	100 ml

Add 25 ml A to 100 ml B

Lugol's iodine solution:

Iodine	1·0 g
Potassium iodide	2·0 g
Distilled/deionised water	300 ml

Safranin:

0·5 % aqueous solution

Hints

- Always use a young culture because older cultures of Gram-positive bacteria tend to lose the ability to retain the crystal violet–iodine complex and appear to be Gram-negative; but some bacteria are naturally only weakly Gram-positive.
- The amount of alcohol treatment (the differential stage) must be judged carefully because over-treatment washes the crystal violet–iodine complex from Gram-positive bacteria and they will appear to be Gram-negative.
- Take care to make an even smear otherwise alcohol will continue to wash the violet/purple colour from thick parts of the smear while thin parts are being over-decolorised.
- At the end of the procedure, check that the labelling has not been washed off by the alcohol.
- Don't despair if the stained smear is not visible to the naked eye; this may happen with a Gram-negative reaction.
- Specimen stained slides are available from suppliers.

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Appendix 1
Safety guidelines

These notes are an updated version of the section on microbiology and biotechnology in Safety in Science Education *(DfEE, 1996) produced as a result of a safety conference of experts (including MISAC representatives) convened by the ASE in 1997. They form Chapter 15 of* Topics in Safety *(3rd Edition, ASE, 2001) and are published with permission.*

General safety considerations

The nature of the growth, reproductive capacity and biochemistry of many micro-organisms 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. Micro-organisms 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.

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 each is provided below.

- **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 be observed in the closed containers in which they were grown.

- **Level 2** (L2): work where there may be 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, sub-culturing or transfer of organisms from one medium to another is not normally done. L2 work may be carried out with pupils between the ages of 11 and 16 years (KS 3n4) and by science teachers who may require training and some supervision, which can be provided through a short in-service course or in school by a knowledgeable biology teacher.

- **Level 3** (L3): work where cultures of known fungi and bacteria are regularly sub-cultured or transferred. This 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.

A significant risk associated with work in microbiology or biotechnology is the generation of microbial aerosols, where fine droplets of water containing cells and/or spores of microbes are released into the air. Aerosols can be formed whenever liquid surfaces are broken 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.

Although microbiology and biotechnology are considered separately in the following sections, they share many safety requirements. However, a major difference is one of scale with a corresponding increase of risk with larger volumes of micro-organisms. Some additional precautions for biotechnology are described.

Before work with microbes is started, pupils should wash their hands with soap and water (except for L2 n L3 work investigating microbes on unwashed hands) and cover any cuts with waterproof plasters. Hands should also always be washed after working with microbes.

Microbiology

Source of Hazard(s)	Guidance
Organisms	L1 Limited to algae, yeasts, moulds and bacteria used for culinary purposes, some moulds and commonly-occurring bacteria where they grow naturally on decaying vegetable material. L2 Care in the choice of suitable cultures (see *Appendix 2: Safe micro-organisms*) must be taken by obtaining them from recognised 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. Organisms may be cultured from the environment but not from environments which are likely to contain harmful organisms, for example, lavatory seats or body surfaces other than fingers or hands. Containers of such cultures, once they have been incubated, must then be sealed before examination. L3 Known cultures from reputable specialist suppliers. Organisms may be cultured from the environment or from body surfaces if the work is appropriate to 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; such as MISAC. 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, in the case of rusts and mildews. 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, blood agar, MacConkey's agar, dung or faecal agar. Similar restrictions apply to broth media. L3 As for L2, unless strict precautions are taken to prevent any release of microbes.
Storage of organisms and media	It is unwise to maintain bacteria and fungi for long periods, in case they become contaminated, except perhaps for some work at L3. Such organisms should be subcultured and checked for purity every 3 months or so but only if aseptic technique can be guaranteed. Mixed cultures of protozoa from reputable suppliers can be maintained indefinitely without risk. 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. Media should be stored as dry powder or tablets. Once sterilised, media can be stored for several months in tightly-sealed, screw-topped bottles away from direct sunlight.
Contamination of teachers, pupils and students	Before beginning practical work, hands should be washed with soap and warm water, and all should be washed again after the activities are finished. There must be no hand-to-mouth operations such as chewing, sucking, licking labels or mouth pipetting. L3 Teachers, technicians and students should wear lab coats or aprons which can be relatively easily disinfected (as necessary) and cleaned. Teachers should consider the use of lab coats or aprons for L2 work.
Inoculation of cultures	Inoculation should involve precautions to prevent contamination of the person and work surfaces. It should also avoid the contamination of culture media with unwanted microbes. Media and Petri dishes, etc. should either be purchased pre-sterilised or sterilised by the user before agar plates are poured. Media must not be deliberately inoculated with material likely to be sources of human pathogens. L3 For the aseptic transfer of cultures, work surfaces should be swabbed with a suitable disinfectant before and after all operations and sufficient time allowed for disinfection to occur. Arrangements should be made to sterilise inoculating loops and spreaders before and after inoculation, and to provide discard pots for pipettes and syringes. The mouths of all containers, tubes, flasks, McCartney bottles etc, 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 little time as possible.

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Source of Hazard(s)	Guidance
Bench surfaces	For practical work by students, benches should be wiped down with a cloth soaked in a suitable disinfectant, preferably before, but always after practical work, and sufficient time allowed for disinfection to occur.
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. Yeast cultures generate considerable quantities of carbon dioxide gas. Incubation containers should be plugged with cotton wool , or closed with plastic caps, or fermentation locks, which will allow excess gas to escape. L2/3 The upper limit for general school-based work should be 30 °C because in this temperature range, (a) cultures of micro-organisms suitable for school use grow well and (b) although some pathogens can grow on certain culture media, there is unlikely to be a hazard when conducting investigations using material derived from suitable environments, e.g., soil, water and appropriate culture media and incubation conditions. Exceptions to this will include yoghurt making (43 °C), the culturing of *Streptococcus thermophilus* (50 °C) and *Bacillus stearothermophilus* (60 °C) and debilitated strains of *E. coli* for work with DNA. Agar plates should be incubated inverted 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. Unless cultures are known to be minimum risk, teachers should consider taping incubated dishes closed around their circumference before examination by pupils or students.
Spills	All spills should be reported to and dealt with by the teacher, who should record all incidents. All spills carry a risk of aerosol formation and procedures for dealing with them must reduce this as far as possible. Spills should be covered with towels or a cloth soaked in a suitable freshly-prepared disinfectant, preferably one that is not appreciably degraded by contact with organic matter or, alternatively, freshly-prepared sodium chlorate(I) (hypochlorite) solution with a concentration preferably greater than 1 %, and left for at least 15 minutes. The spill debris should then be swept up using disposable paper towels. Disposable plastic gloves should be worn. Seriously contaminated clothing should be disinfected before laundering. Contaminated skin should be carefully washed with soap and hot water. Lysol and other cresolic disinfectants are caustic and toxic by skin absorption and should not be used. Clear phenolics are suitable but are no longer so readily available to schools in sensible quantities at reasonable prices. MicroSol (Virkon) is a relatively stable alternative. To prevent breakages and spills, cultures must be centrifuged in capped, plastic tubes.
Observation of cultures of bacteria and fungi	L1 Cultures should be viewed in the unopened containers in which they were grown. L2 Cultures should be examined by pupils in containers which have been taped closed. If cultures may contain pathogens and there is a risk that students may open them, even though instructed not to do so, it will be prudent for the cultures to be completely sealed with tape before examination. If it is necessary for pupils to open cultures for examination, special precautions may be necessary. Other than for pure, non-pathogenic cultures prepared by teachers or technicians under aseptic conditions, and especially for cultures originating from environmental samples in which pathogens may be present, these must be killed by the teacher or technician as follows. A filter paper is placed in the lid of an inverted agar plate and moistened with 40 % methanal solution (formalin). After 24 hours the filter paper is removed. (Take care with methanal: eye protection, gloves and use of a fume cupboard to avoid breathing fumes are necessary). 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 described above.
Sterilisation and disposal	All cultures must be heated to kill micro-organisms before disposal. This is best done using a pressure cooker or autoclave, in conjunction with autoclavable bags. The caps of all screw-topped bottles must be loosened before cultures and media are sterilised. It is very important that instructions for use of the auto clave are followed in order to achieve and maintain sufficiently high temperatures for a long enough time. Pressure cookers are unlikely to be equipped with appropriate instructions for sterilisation and those for some autoclaves, designed for use with surgical instruments, state that the equipment is unsuitable for

Source of Hazard(s)	Guidance
	sterilising liquid media. Such autoclaves can be used for microbiological preparations but advice on their correct operation should be sought. Teachers and technicians should be trained to follow safe working practices. Seals and safety valves should be checked before each use. Heating autoclaves or pressure cookers with Bunsen burners is not recommended. Rapid cooling and the release of steam to lower the internal pressure quickly to atmospheric pressure is dangerous because it may shatter glassware and/or cause liquid media to boil over. Equipment should be allowed to cool unaided before opening. Further information may be sought from the advisory bodies such as MISAC. Sterilisation cannot be achieved by the use of chemical disinfectants. If, in exceptional circumstances, chemical disinfection of cultures is contemplated prior to disposal, use a freshly-made solution of a disinfectant that is not degraded when in contact with organic matter (see 'Spills' above). Cultures and equipment must be opened under the surface of the solution and left for at least 12 hours. Again it is essential to follow disinfectant instructions carefully. Chlorate(I) solution is inactivated by large amounts of organic matter, although if a culture might contain viruses this is often the preferred disinfectant. Microwave ovens are not suitable for sterilisation of most items, though they are sometimes used for liquefying prepared agar media. Beware of explosions which have occurred when solid medium has blocked the opening of a container. After sterilisation, solid cultures can be disposed of, in tied autoclave bags or similar, through the refuse system; liquid cultures can be flushed away down the lavatory or the sink with lots of water. Sterilised culture material should not be allowed to accumulate in open or closed waste traps. Incineration is an acceptable alternative to autoclaving. (Note, however, that polystyrene Petri dishes will generate harmful fumes when incinerated; a purpose-built incinerator with a tall flue should be used.) Clean glass equipment can be sterilised by dry heat in an oven (165 °C for at least 2 hours) or, in the case of wire loops, by heating to red heat in a Bunsen burner flame.
Radiation	Use to induce mutations in yeasts only; ensure that eyes are protected from UV radiation.

Biotechnology

For guidance on practical work with DNA, please consult the NCBE (www.ncbe.reading.ac.uk).

Source of Hazard(s)	Guidance
Organisms	The level restrictions which apply for microbiology also apply to biotechnology work. L1 Particularly suitable organisms include yoghurt bacteria, yeasts such as for the production of wine or bread and some unicellular algae. L2 Other than L1 organisms, it is recommended that organisms with unusual growth requirements are used, e.g., high salt, low pH, low temperature. Some examples of minimum risk organisms include *Vibrio natriegens* (*Beneckea natriegens*), *Photobacterium phosphoreum* and *Acetobacter aceti*. Avoid the large-scale culture of organisms which produce antibiotics, particularly penicillin. Cultures of organisms should only be obtained from recognised suppliers, including culture collections. L3 As for L2.
Culture media	The solutions generally used in biotechnology work present few problems other than those associated with quantity and the potential for contamination. Scaling up is often a necessary activity in biotechnology but keep quantities to a sensible minimum to make handling easier and reduce the quantities of enzymes, antibiotics etc. which may be generated. All media should be sterilised prior to use. The use of animal dung for investigations of biogas generation is not recommended; use grass clippings inoculated with well-rotted garden compost.
Incubation/fermentation	To minimise the risks from contaminating pathogenic organisms, incubation temperatures should be no more than 30 °C. However, for yoghurt making, 43 °C may be used if hygienic preparation is followed and for work with DNA using K12 strains of *E. coli*, incubation at 37 °C is permissible assuming good aseptic technique. L2/3 Use of fermenters is limited to these levels.

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	The generation of large volumes of gas (carbon dioxide or methane) is a risk associated with many fermentations. Vessels must be suitably vented to allow the gas to escape but prevent aerosol formation or the entry of unwanted organisms. In the case of methane, the gas must be kept away from naked flames and electrical equipment which can cause sparks. Other than for work with yeasts and small-scale biogas generation using plant material, wholly anaerobic fermentations should not be used in schools. Investigations which are partially anaerobic, e.g. setting up a Winogradsky column, may, however, be attempted.
Contamination	Cultures should be started by inoculation with a significant volume of actively-growing inoculum (for example 20 % of total volume). All equipment and materials (other than the inoculum) should be sterilised prior to use.
Spills	Routines for dealing with spills are the same as for microbiology. With fermenters there is the risk of spills of large amounts of liquid culture. All possible steps should be taken to guard against this, for example, by using equipment within a spills tray. In the case of gross spills, unless the organism is known to be safe, the lab should be cleared before attempting to deal with the spill.
Electrical hazards	Keep all electrical leads, especially mains leads, tidy and site electrical equipment so as to minimise the risk of water entering.
Disposal	All cultures should be sterilised before disposal, preferably in an autoclave. If a fermenter cannot be sterilised complete, add a freshly-prepared disinfectant that is not appreciably degraded by contact with organic matter to the culture and leave for sufficient time to enable disinfection to occur before pouring the contents into containers which can be autoclaved.
Enzymes	Handle all enzymes, whether solid or liquid, or cultures which may produce them, with due care. Problems with enzymes increase with quantity as well as variety. Minimise skin contact and use eye protection and disposable gloves for solid or concentrated solutions of lipolytic and proteolytic enzymes. Avoid the release of powders into the air.

Appendix 2
Safe micro-organisms

In May 1997, a safety conference was convened by the Association for Science Education (ASE). Various organisations were represented, including the ASE, CLEAPSS, SSERC, HSE, MISAC (Microbiology in Schools Advisory Committee), Society for Applied Microbiology, Society for General Microbiology, NCBE (National Centre for Biotechnology Education), SAPS (Science n Plants in Schools), the Wellcome Trust and the educational suppliers Philip Harris and Blades Biological. The principal aims of the conference were to consider clarification of guidance on the use of micro-organisms and biotechnology in the DfEE publication *Safety in Science Education* and to evaluate the list of micro-organisms considered suitable for use in schools and colleges following changes to the hazard categorisation of certain micro-organisms by the Advisory Committee on Dangerous Pathogens[1]. One of the outcomes of the conference is a revision of this list.

The accompanying tables give selected micro-organisms which present minimum risk given good practice. These tables supersede the existing lists found in the *CLEAPSS Laboratory Handbook* (1992), the *CLEAPSS Shorter Laboratory Handbook* (2000), *Microbiology: An HMI Guide for Schools and Further Education* (1990, now out of print), *Topics in Safety* (1988) and *Safety in Science Education* (1996). As well as naming suitable organisms, the new lists give points of educational use and interest and comment on the ease with which organisms can be cultured and maintained. The lists of micro-organisms are not definitive; other organisms may be used if competent advice is obtained.

It should be noted that strains of micro-organisms can differ physiologically and therefore may not give expected results. Where possible, fungi that produce large numbers of air-borne spores should be handled before sporulation occurs, so that the spread of spores into the air and possible risks of allergy or the triggering of asthmatic attacks are minimised. This is particularly important for some species, such as *Aspergillus* and *Penicillium*, which produce very large numbers of easily dispersed spores. It should be noted that certain species of these two fungi, previously listed as unsuitable for use in schools, are now not thought to present such a serious risk to health, given good practice in culture and handling.

[Note: This list of micro-organisms is also be available from other sources including the ASE, CLEAPSS, MISAC and SSERC and is published in the new edition of Topics in Safety *(ASE, 2001, ISBN 0 86357 316 9) with notes on other aspects of safety relating to microbiology and biotechnology in schools.]*

[1]*Categorisation of Biological Agents According to Hazard and Categories of Containment*, 4th edition, 1995, Advisory Committee on Dangerous Pathogens, HSE Books, ISBN 0717610381.

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Bacteria

Bacterium	Educational use/interest/suitability	Ease of use/maintenance
Acetobacter aceti	Of economic importance in causing spoilage in beers and wines. Oxidises ethanol to ethanoic (acetic) acid and ultimately to carbon dioxide and water.	Needs special medium and very frequent subculturing to maintain viability.
Agrobacterium tumefaciens	Causes crown galls in plants; used as a DNA vector in the genetic modification of organisms.	Grows on nutrient agar, but requires 2-3 days' incubation.
Alcaligenes eutrophus	In the absence of nitrogen, it produces intracellular granules of poly-β-hydroxybutyrate (PHB); was used in the production of biodegradable plastics.	Grows on nutrient agar.
Azotobacter vinelandii	A free-living nitrogen fixer, producing a fluorescent, water-soluble pigment when grown in iron (Fe)-limited conditions.	Grows on a nitrogen-free medium.
Bacillus megaterium	Has very large cells; produces lipase, protease and also PHB (see *Alcaligenes*); Gram-positive staining.	Grows on nutrient agar.
Bacillus stearothermophilus	Thermophilic species which grows at 65 °C; produces lipase and protease. Also used to test the efficiency of autoclaves.	Grows on nutrient agar.
*Bacillus subtilis**	General-purpose, Gram-positive bacterium. Produces amylase, lipase and protease.	Grows on nutrient agar.
Cellulomonas sp.	Produces extracellular cellulase.	Grows on nutrient agar but also used with agar containing carboxymethylcellulose.
Chromatium sp.	A photosynthetic, anaerobic bacterium.	Requires special medium and light for good growth.
Erwinia carotovora (=*E. atroseptica*)	Produces pectinase which causes rotting in fruit and vegetables. Useful for studies of Koch's postulates.	Grows on nutrient agar.
*Escherichia coli**	K12 strain: general-purpose, Gram-negative bacterium. B strain: susceptible to T4 bacteriophage.	Grows on nutrient agar.
Janthinobacterium (=*Chromobacterium*) *lividum*†	Produces violet colonies. Grows best at 20 °C.	Needs frequent subculture and is best grown on glucose nutrient agar and broth.
Lactobacillus sp.	Ferment glucose and lactose, producing lactic acid; *L. bulgaricus* is used in the production of yoghurt.	Require special medium containing glucose and yeast extract and frequent subculturing to maintain viability.
Leuconostoc mesenteroides	Converts sucrose to dextran: used as a blood plasma substitute.	Requires special medium as for *Lactobacillus*.
Methylophilus methylotrophus	Requires methanol as energy source; was used for the production of 'Pruteen' single-cell protein.	Requires special medium containing methanol.
Micrococcus luteus (=*Sarcina lutea*)	Produces yellow colonies; useful in the isolation of the bacterium from impure cultures. Also used to simulate the effects of disinfectants, mouthwashes and toothpastes on more harmful organisms. General-purpose, Gram-positive bacterium.	Grows on nutrient agar.
Photobacterium phosphoreum	Actively-growing, aerated cultures show bioluminescence; grows in saline conditions.	Requires a medium containing sodium chloride.
Pseudomonas fluorescens	Produces a fluorescent pigment in the medium.	Grows on nutrient agar.
Rhizobium leguminosarum	A symbiotic, nitrogen fixer; stimulates the formation of nodules on the roots of legumes. Only fixes nitrogen in plants.	Grows on yeast malt agar; some authorities recommend buffering with chalk to maintain viability.
Rhodopseudomonas palustris	A photosynthetic, anaerobic, red bacterium. Also grows aerobically in the dark.	Requires light and a special medium, growing atypically on nutrient agar.

Bacterium	Educational use/interest/suitability	Ease of use/maintenance
Spirillum serpens	Of morphological interest.	May grow on nutrient agar but requires very frequent subculturing to maintain viability.
Staphylococcus albus (*epidermidis*)‡	A general-purpose, Gram-positive bacterium, producing white colonies.	Grows on nutrient agar.
Streptococcus (=*Enterococcus*) *faecalis*	Of morphological interest, forming pairs or chains of cocci.	Nutrient agar with added glucose can be used but grows better on special medium, as for *Lactobacillus*.
Streptococcus (=*Lactococcus*) *lactis*	Of morphological interest, forming pairs or chains of cocci. Commonly involved in the souring of milk; also used as a starter culture for dairy products.	Can grow on nutrient agar with added glucose; some authorities recommend buffering with chalk to maintain viability.
Streptococcus thermophilus	Ferments glucose and lactose, producing lactic acid; used in the production of yoghurt. Grows at 50 °C.	Can grow on nutrient agar with added glucose; some authorities recommend frequent subculturing to maintain viability.
Streptomyces griseus	Responsible for the earthy odour of soil. Grows to form a fungus-like, branching mycelium with aerial hyphae bearing conidia. Produces streptomycin.	Grows on nutrient or glucose nutrient agar but better on special medium which enhances formation of conidia.
Thiobacillus ferrooxidans	Involved in the bacterial leaching of sulphur-containing coal. Oxidises iron(II) and sulphur. Demonstrates bacterial leaching of coal samples containing pyritic sulphur.	Requires special medium.
Vibrio natriegens§ (=*Beneckea natriegens*)	A halophile, giving very rapid growth. Prone, however, to thermal shock with a sudden drop in temperature.	Requires medium containing sodium chloride.

*Some strains have been associated with health hazards. Reputable suppliers should ensure that safe strains are provided.

†Can be chosen for investigations that once required the use of *Chromobacterium violaceum* or *Serratia marcescens*.

‡This organism has been known to infect debilitated individuals and those taking immunosuppressive drugs. Some authorities advise against its use.

§A well-known supplier currently lists an unspecified species of *Vibrio* because of its morphological interest. This has a typical shape, better shown than by *V. natriegens*. However, this species is a Hazard Group 2 organism which may cause human disease. This bacterium should only be used in establishments that have containment facilities suitable for work with Hazard Group 2 micro-organisms.

Fungi

Fungus	Educational use/interest/suitability	Ease of use/maintenance
Agaricus bisporus	Edible mushroom; useful for a variety of investigations on factors affecting growth.	Grows on compost containing well-rotten horse manure; available as growing 'kits'.
Armillaria mellea	The honey fungus; causes decay of timber and tree stumps. Produces rhizomorphs.	Grows very well on malt agar. Some authorities recommend carrot agar.
*Aspergillus nidulans**	For studies of nutritional mutants. Produces abundant, easily-dispersed spores – may become a major laboratory contaminant!	Grows on Czapek Dox yeast agar. Special media required for studying nutritional mutants.
*Aspergillus niger**	Useful for studies of the influence of magnesium on growth and the development of spore colour. Used commercially for the production of citric acid. Produces abundant, easily-dispersed spores - may become a major laboratory contaminant!	Requires special sporulation medium for investigations.
*Aspergillus oryzae**	Produces a potent amylase; useful for studies of starch digestion. Also produces protease. Used by the Japanese in the production of rice wine (saki).	Grows on malt agar; add starch (or protein) for investigations.

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Fungus	Educational use/interest/suitability	Ease of use/maintenance
Botrytis cinerea	Causes rotting in fruits, particularly strawberries. Useful for studies of Koch's postulates with fruit, vegetables and *Pelargonium* spp. Important in the production of some dessert wines ('noble' rot). Used in ELISA protocols.	Can be grown on malt agar or agar with oatmeal.
Botrytis fabae	Causes disease in bean plants.	Requires agar with oatmeal.
Candida utilis	Simulates behaviour of pathogenic *Candida* spp. in investigations of fungicidal compounds.	Grows on malt agar or glucose nutrient agar.
Chaetomium globosum	Useful for studies of cellulase production; thrives on paper.	Can be grown on V8 medium but survives well just on double thickness wall paper, coated with a flour paste.
Coprinus lagopus	For studies of fungal genetics.	Grows on horse dung.
Eurotium (=*Aspergillus*) *repens*	Produces yellow cleistocarps (cleistothecia) embedded in the medium and green conidial heads in the same culture.	Requires special medium.
Fusarium graminearum	Causes red rust on wheat; used in the manufacture of 'Quorn' mycoprotein.	Can be grown on V8 medium.
Fusarium oxysporum	A pathogen of many plants. Produces sickle-cell-shaped spores, a red pigment and pectinase.	Grows well on several media including malt, potato dextrose and Czapek Dox yeast agar.
Fusarium solani	Digests cellulose; macroconidia have a sickle shape.	Grows on potato dextrose agar.
Helminthosporium avenae	A pathogen of oats.	May not grow easily in laboratory cultures.
Kluyveromyces lactis	A yeast, isolated from cheese and dairy products. Ferments lactose and used to convert dairy products to lactose-free forms. Genetically-modified strains are used to produce chymosin (rennet).	Grows on malt agar or glucose nutrient agar.
Leptosphaeria maculans	For studies of disease in *Brassica* plants.	Requires cornmeal agar or prune yeast lactose agar to promote sporulation in older cultures.
Monilinia (=*Sclerotinia*) *fructigena*	For studies of brown rot in apples. Useful for studies of Koch's postulates.	Grows on malt agar or potato dextrose agar.
Mucor genevensis	For studies of sexual reproduction in a homothallic strain of fungus.	Grows on malt agar.
Mucor hiemalis	For studies of sexual reproduction between heterothallic + and – strains and zygospore production.	Grows on malt agar.
Mucor mucedo	Common black 'pin mould' on bread. For sporangia (asexual), mating types and amylase production.	Grows on malt agar.
Myrothecium verucaria	For studies of cellulose decomposition, but *Chaetomium globosum* is preferred.	Grows on malt agar.
*Neurospora crassa**	Red bread mould. Produces different coloured ascospores. Can be used in studies of genetics. Beware – readily becomes a major laboratory contaminant!	Grows on malt agar.
*Penicillium chrysogenum**	Produces penicillin; useful for comparative growth inhibition studies in liquid media or when inoculated on to agar plates seeded with Gram-positive and -negative bacteria. Produces yellow pigment.	Grows on malt agar, though some authorities indicate that it thrives better on liquid media.
*Penicillium expansum**	Does not produce penicillin; causes disease in apples. Useful for studies of Koch's postulates.	Grows on malt agar.
*Penicillium notatum**	Produces penicillin; useful for comparative growth inhibition studies in liquid media or when inoculated onto agar plates seeded with Gram-positive and -negative bacteria.	Grows on malt agar.

Fungus	Educational use/interest/suitability	Ease of use/maintenance
*Penicillium roqueforti**	Does not produce penicillin; the familiar mould of blue-veined cheese.	Grows on malt agar.
*Penicillium wortmanii**	Produce wortmin rather than penicillin.	Grows on malt agar.
Phaffia rhodozyma	A fermenting red yeast. Used to colour the food supplied to fish-farmed salmon.	Grows on yeast malt agar.
Phycomyces blakesleanus	Produces very long sporangiophores which are strongly phototropic.	Grows on malt agar.
Physalospora obtusa	An ascomycete fungus that grows on apples. Thought to produce pectinase.	Grows on potato dextrose agar.
Phytophthora infestans†	Causes potato blight. Produces motile zoospores.	Can be grown on V8 medium.
Plasmodiophora brassicae	For studies of disease in *Brassica* plants, particularly club root. Useful for studies of Koch's postulates.	May not grow easily in culture.
Pleurotus ostreatus	Edible oyster cap mushroom.	Can be grown on rolls of toilet paper!
Pythium de baryanum†	Causes 'damping off' of seedlings; cress is best to use.	Grows on cornmeal agar.
Rhizopus oligosporus	Used in the fermentation of soya beans to make 'tempe', a meat-substitute food in Indonesia.	Grows on potato dextrose agar, Czapek Dox yeast agar and other fungal media.
Rhizopus sexualis	Produces rhizoids and zygospores. Useful for studies of the linear growth of fungi.	Grows on potato dextrose agar and other fungal media.
Rhizopus stolonifer	Produces rhizoids. Produces lipase.	Grows on potato dextrose agar, potato carrot agar, Czapek Dox yeast agar and other fungal media.
Rhytisma acerinum	An indicator of air pollution: less common in industrial areas. On sycamore leaves, it forms 'tar' spot lesions, the number or diameter of which can be compared at different sites.	Difficult to maintain but laboratory cultures are not likely to be needed.
Saccharomyces cerevisiae	Valuable for work in baking and brewing, showing budding, for spontaneous mutation and mutation-induction experiments, and for gene complementation using adenine- and histidine-requiring strains.	Grows on malt agar or glucose nutrient agar.
Saccharomyces diastaticus	Able to grow on starch by producing glucoamylase.	Grows on malt agar and nutrient agar + 1 % starch.
Saccharomyces ellipsoideus	Used in fermentations to produce wine; can tolerate relatively high concentrations of ethanol.	Grows on malt agar.
Saprolegnia litoralis†	Parasitic on animals. Produces zoospores. Good illustration of asexual and sexual stages.	Culture by baiting pond water with hemp seeds.
Schizosaccharomyces pombe	Large cells, dividing by binary fission. Good for studies of population growth, using a haemocytometer for cell counts. Prone to thermal shock.	Grows on malt agar. For studies of population growth, a malt extract broth can be used.
Sordaria brevicollis	For studies of fungal genetics, including inheritance of spore colour and crossing over in meiosis.	Requires special medium for crosses between strains.
Sordaria fimicola	For studies of fungal genetics, including inheritance of spore colour and crossing over in meiosis.	Grows on cornmeal, malt and other agars but may not transfer readily from one medium to another. White-spore strain may not always grow normally on standard cornmeal agar.
Sporobolomyces sp.	Found on leaf surfaces. Spores are ejected forcibly into the air from mother cells.	Grows on malt, yeast malt and glucose nutrient agar but laboratory cultures may not be needed.
Trichoderma reesei	Commercial production of cellulase.	Grows on malt agar.

*Possible risk of allergy/asthma if large numbers of spores are inhaled.

†Now classed as a protoctist, so may not be listed under fungi by some suppliers.

Viruses

These are rarely used in schools and colleges but a selected list of those which might be considered is given below.

Bacteriophage (T type) (host *E. coli*)	Cucumber Mosaic Virus
Potato Virus X	Potato Virus Y (*not the virulent strain*)
Tobacco Mosaic Virus	Turnip Mosaic Virus

Algae, protozoa (including slime moulds) and lichens

Though some protozoa are known to be pathogenic, the species quoted for experimental work in recent science projects and those obtained from schools' suppliers or derived from hay infusions, together with species of algae and lichens, are acceptable for use in schools.

Unsuitable micro-organisms

A number of micro-organisms have in the past been suggested for use in schools but are no longer considered suitable; these are listed below. Some fungi previously considered unsuitable have been reinstated in the list of selected organisms now that it is thought that they do not present a major risk, given good practice.

Bacteria	Fungi
Chromobacterium violaceum	*Rhizomucor* (*Mucor*) *pusillus*
Clostridium perfringens (*welchii*)	
Pseudomonas aeruginosa	
Pseudomonas solanacearum	
Pseudomonas tabaci	
Serratia marcescens	
Staphylococcus aureus	
Xanthomonas phaseoli	

Appendix 3
Safety resources

There is no specialist published guide wholly devoted to safety in practical microbiology in schools still in print; this appendix lists some relevant resources, together with the contact details of organisations which can be approached for advice.

Publications

This book is recommended as containing the most up-to-date and detailed information on the safe use of micro-organisms in schools:

Topics in Safety, 3rd edition (ASE, 2001) – Basic health and safety management in schools. Chapter 15 – Microbiology n biotechnology – contains detailed information, includes risks associated with each procedure; levels of work, list of suitable and unsuitable micro-organisms, steam sterilisation, sub-culturing and transfer work at level 3.

Other titles

CLEAPSS Science Publications, CD-ROM (latest edition)

Safetynet (SSERC).

Safeguards in the School Laboratory, 11th edition (ASE 2006) – General safety guidance. Health n Safety legislation, managing safety, GLP, use of pressure vessels, section on biological hazards includes microbiology/biotechnology.

Safety in Science Education (DfEE, HMSO, 1996, available from the ASE website – www.ase.org.uk) – Comprehensive guide to safety. Includes legislation, safety management in the science department, risk assessments, emergency procedures, first aid, resources, laboratory design, GLP, use of chemicals, use of electricity. Section 17.4 on microbiology is inaccurate and has been superseded by Chapter 15 in the new edition (2001) of Topics in Safety (see above).

Sources of Advice

MISAC – www.misac.org.uk

CLEAPSS – www.cleapss.org.uk

SSERC – www.sserc.org.uk

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Appendix 4

Suppliers of cultures and equipment to schools and colleges

The following companies supply cultures and/or equipment for practical microbiology in schools and colleges. Inclusion in the list implies no recommendation. Before purchasing cultures, please refer to *Appendix 2: Safe micro-organisms* to ensure that the organism is suitable for use in schools.

Company	Address	Contact details	Products
Beecroft and Partners Ltd	Northfield Road Rotherham South Yorkshire S60 1RR	Tel. 01709 377881 Email sales@beecroft-science.co.uk www.beecroft-science.co.uk	Fungi and bacteria; culture media and antibiotic discs
Blades Biological	Cowden Edenbridge Kent TN8 7DX	Tel. 01342 850242 Email sales@blades-bio.co.uk www.blades-bio.co.uk	Algae, protozoa, fungi and bacteria; culture media and antibiotic discs
National Centre for Biotechnology Education (NCBE)	University of Reading 2 Earley Gate Whiteknights Reading RG6 6AU	Tel. 0118 987 3743 Email ncbe@reading.ac.uk www.ncbe.reading.ac.uk	Limited range of cultures of bacteria and fungi; items for microbiology and molecular biology; microbiology kits
Philip Harris	Hyde Buildings Ashton Road Hyde Cheshire SK14 4SH	Tel. 0845 120 4520 Email orders@philipharris.co.uk www.philipharris.co.uk	Full range of cultures, media and equipment for practical microbiology
Sciento	61 Bury Old Road Whitefield Manchester M45 6TB	Tel. 0161 773 6338 Email sales@sciento.co.uk www.sciento.co.uk	Algae, protozoa, fungi, and bacteria; culture media and antibiotic discs
Timstar	Unit 12 Ion Path Road Three Winsford Industrial Estate Winsford Cheshire CW7 3BX	Tel. 01270 250459 Email sales@timstar.co.uk www.timstar.co.uk	Full range of cultures, media and equipment for practical microbiology

Appendix 5

Use of the autoclave/pressure cooker

General

- Autoclaves have a larger capacity than pressure cookers.
- Pressure cookers are less expensive, are lighter, have a shorter sterilisation cycle time and are easier to maintain than autoclaves.
- Pressure cookers with a high dome have greater capacity than those without.
- Pressure cookers with weights to control steam pressure enable the pressure to be varied.
- Sterilisation is achieved in an atmosphere of steam under pressure.
- Usual conditions are not less than 15 minutes exposure to pure steam at a pressure of 103 kPa (kN m^2) or 15 lbf in^{-2}, producing a temperature of 121 °C.

Hint

Label the vessels to be sterilised with a permanent marker pen or write on the greaseproof paper that covers a cotton wool plug (see below).

Loading

- Do not overload otherwise there will not be sufficient space for steam to circulate.
- Limit the volume of material to be sterilised to ca 250 cm^3 per vessel to allow rapid and thorough penetration of heat (larger volumes may require a longer exposure time).
- Fill vessels to no more than $^2/_3$ capacity and loosen caps of screw-capped vessels to allow for expansion.
- Protect cotton wool plugs from becoming wet and losing their ability to function as a barrier to the passage of airborne microbes by covering with either greaseproof paper or aluminium foil.

Steam generation

- Ensure that there is more than enough water to generate steam throughout the sterilisation cycle and to prevent boiling dry.
- Use a gas ring or hot plate, not Bunsen burners and a tripod.
- Use either distilled or deionised water to prevent corrosion.

Sterilisation

- Allow several minutes of vigorous release of steam to ensure complete expulsion of air otherwise the required temperature will not be achieved.
- Begin timing the holding time when air has been completely expelled and the heat has penetrated throughout the materials to be sterilised.
- Ensure that steam continues to escape throughout the holding time.

Unloading

- After completion of the holding time, allow the autoclave/pressure cooker to return atmospheric pressure and temperature before starting the opening procedure.
- Never attempt to reduce either the cooling period with cold water or the time taken for pressure reduction by premature opening of the air cock.

After care

- Clean out, empty and thoroughly dry the autoclave/pressure cooker to prevent corrosion.
- Correct use, maintenance and regular examination (as required by the Pressure Systems Safety Regulations) of autoclaves and pressure cookers will ensure that risk of malfunction (explosions and failure to achieve sterilisation) is virtually eliminated.

Contact CLEAPSS for further detailed guidance on using autoclaves and pressure cookers (Tel. 01895 251496; Email science@cleapss.org.uk; www.cleapss.org.uk).

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Appendix 6

Preparing serial dilutions

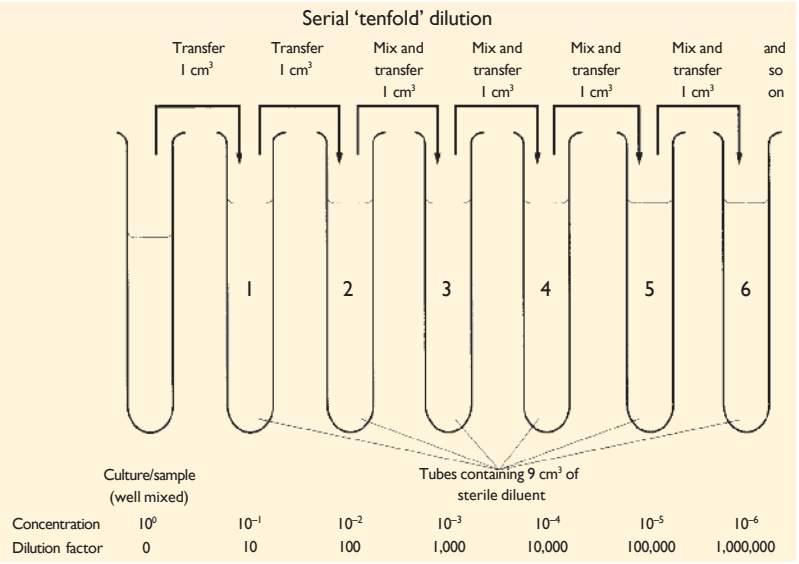
There are various ways of counting or monitoring microbial growth in a culture.

Serial dilution involves taking a sample and diluting it through a series of standard volumes of sterile diluent, e.g. distilled water or 0·9 % saline. Then a small measured volume of each dilution is used to make a series of pour or spread plates.

By diluting the sample in this controlled way it is possible to obtain an incubated plate with an easily countable number of colonies (30–100) and calculate the number of microbes present in the sample.

Materials

- Culture of bacteria or yeast or sample of natural material
- 6 sterile test tubes containing 9 cm^3 sterile diluent, fitted with a cap or cotton wool plug, labelled 1→6 and with the dilution factor as shown in the diagram
- 12 sterile, plugged Pasteur pipettes
- 1 cm^3 syringe barrel fitted with rubber tubing (see page 9)
- Pot of Virkon disinfectant



Procedure

1. Take a sterile pipette.
2. Place the syringe onto the plugged end of the pipette (see page 9).
3. Draw up 1 cm^3 of a well mixed sample/culture into the pipette.
4. Add this sample to the first tube. The volume of this tube is now 10 cm^3. This provides an initial dilution of 10^{-1}.
5. Mix the dilution thoroughly, by emptying and filling the pipette several times.
6. Discard this pipette into the pot of disinfectant, but keep the syringe for making the next dilution.
7. Take a new pipette, fit it to the syringe and draw up a 1 cm^3 sample of the 10^{-1} dilution and place it in the second tube.
8. Mix well as before. This gives a 10^{-2} dilution.
9. Discard the pipette in disinfectant.
10. Repeat this for the remaining tubes, removing 1cm^3 from the previous dilution and adding it to the next 9 cm^3 of diluent.

If 6 tubes are used, the final dilution for the bacteria will be 10^{-6} (1 in 1,000,000).

Plating and counting procedure

Use a known volume of each dilution to make either pour plates (page 12) or spread plates (page 14). By starting with the highest dilution, the same pipette may be used throughout. For statistical purposes, replicate plates should be prepared.

After incubation the plates will show a range of numbers of colonies. Choose the plate that has an easily countable number (about 30–100) and carefully count every colony. Using a marker pen helps to avoid counting the same colony twice. Then calculate the number of micro-organisms in the sample:

Number of microbes/cm^3 = number of colonies × dilution of sample

Microbiology Society

The Microbiology Society is a membership organisation for scientists who work in all areas of microbiology. It is the largest learned microbiological society in Europe with a worldwide membership based in universities, industry, hospitals, research institutes and schools. The Society publishes key academic journals, organises international scientific conferences and provides an international forum for communication among microbiologists and supports their professional development. The Society promotes the understanding of microbiology to a diverse range of stakeholders, including policy-makers, students, teachers, journalists and the wider public, through a comprehensive framework of communication activities and resources.

The Society produces and distributes a wide range of resources including videos to support microbiology teaching in schools and colleges. The Society offers membership to schools or school representatives, runs courses and offers an information service to teachers.

For tutorial videos visit: https://www.youtube.com/c/MicrobiologySocietyOrg1945. For more information, see www.microbiologyonline.org.uk or contact the Education Department, Microbiology Society, Charles Darwin House, 12 Roger Street, WC1N 2JU, London, UK (education@microbiologysociety.org.uk).

Micro-organisms, bacteria, archaea, viruses, fungi, protozoa and algae are everywhere, and their activities are vitally important to virtually all processes on Earth. They play key roles in nutrient cycling, biodegradation/biodeterioration, global warming, food spoilage, the cause and control of disease, and biotechnology. Thanks to their versatility, microbes can be put to work in many ways: making life-saving drugs; the manufacture of biofuels; cleaning up pollution; and producing/processing food and drink.

This manual explains the basic techniques necessary to carry out microbiology experiments safely and ettectively, and is intended as a guide for teachers and technicians. This manual can be used in conjunction with the book *Practical Microbiology for Secondary Schools*, also produced by the Microbiology Society, and supports the National Curriculum and equivalent qualifications in schools and colleges.

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