Safe Handling and Maintenance of Micro Organisms

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Introduction

There are many experiments in microbiology and biotechnology suitable for use in schools and colleges. These can be carried out without risk to teachers, technicians or pupils provided that appropriate handling techniques are used.

This booklet sets out to provide the correct safety guidelines and appropriate procedures to be used when handling micro organisms supplied by Philip Harris in schools and non advanced further education.

We constantly review our listing of organisms to ensure that we only supply organisms currently considered suitable for school/college use. It is however important that individual teachers are familiar with the recommendations of Her Majesty's Inspectors and follow the guidelines set down for pupils of different ages.

They should also be adequately trained in appropriate procedures for the handling and disposal of micro-organisms.

General Safety Rules

The laboratory

Laboratories and, in particular, preparation rooms where microorganisms are being handled require a few basic features to promote safe working practice.These are:-

- Bench surfaces should be impervious to water and resistant to acids, alkalis, solvents and disinfectants.
- A sink must be available for hand washing.
- An autoclave for the sterilisation of waste materials should be available (see section on Disposal).

Procedures

When carrying out microbiological work the laboratory door and windows should be closed to avoid draughts.

As well as following normal procedures for laboratory safety, the following points must be adhered to by teachers, technicians and students.

1. Wear a protective coat.
2. Wash hands and cover any cuts with waterproof dressings.
3. Avoid all hand to mouth operations (e.g. licking labels) and do not eat or drink in the laboratory.
4. Follow the correct aseptic techniques when transferring organisms (refer to Aseptic Techniques).
5. Provide a container of 2% sodium hypochlorite solution for the disposal of used pipettes, culture vessels etc. (refer to Disposal).
6. Do not isolate cultures from potentially dangerous sources such as human mucus, cuts etc. Similarly, do not subculture unknown organisms - they may be pathogens.
7. Swab spillages immediately.
8. Label cultures and seal for incubation with adhesive tape. It it is necessary to open the cultures after incubation, a filter paper disc soaked in 40% formalin should be placed in the lid of the dish 24 hours prior to examination.
9. When the work is completed, dispose of all contaminated equipment as indicated in point 5 above. Swab the bench, remove coat and wash hands before leaving the laboratory.
10. While cultures can be kept in a refrigerator to increase their 'life', care should be taken that such laboratory refrigerators are not used to store food for human or animal consumption.

Aseptic Techniques

The techniques described here have been developed to prevent contamination of cultures by foreign organisms and to prevent the contamination of operators by the organisms being handled.

It is important to understand that the organisms recommended as suitable for use in schools and colleges are considered non-pathogenic to humans. However, all such organisms should be treated as if they are pathogenic to prevent accidental infection from any mutants or contaminants that might have been inadvertently introduced.

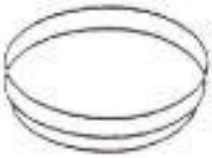
To prevent any such infection from occurring it is also important to understand how infections might occur in the laboratory. Infection may occur in the following ways:

Method of infection	Remedy
INHALATION of aerosols (i.e. water droplets Containing bacteria or fungal spores). These are formed when culture vessels are opened carelessly and organisms transferred incorrectly.	Use aspectic techniques.
SKIN CONTACT- as a result of breakages or spillages.	Swab with antiseptic (see 'disposal').
INGESTION – as a result of pipetting cultures by mouth.	Never pipette orally, always use teat pipettes.

Bacteria and fungi are generally grown on a special medium in a screw topped universal container or a McCartney bottle or petri dish. These containers are transparent and allow the growth of the colony to be observed. The micro-organisms are handled by means of an inoculating loop.

Inoculating loop

McCartney bottle



Petri dish

Bacteria and fungal spores are in the air around us, on our clothes and bodies, in dust, etc., and it is therefore important to follow set procedures when handling cultures of micro-organisms. These procedures have been designed to minimise the risk of contamination both from, and to, the environment and are generally known as 'aseptic' techniques. Techniques should be practised several times as a 'dry-run' before working with cultures, so that any initial awkwardness can be overcome.

A bunsen burner or spirit burner is required for the sterilisation of the inoculating loop unless disposable loops are used.

Preparations for working aseptically:

1. Close doors and windows. Select an area of bench away from draughts.
2. Swab the bench top with 70% alcohol.
3. Assemble the apparatus required.
4. Work near a bunsen or spirit burner as the up draught from the flame will help to prevent contamination.

Note: A suitable transfer chamber may be used instead of the open bench. The Harris Transfer Chamber has been specially designed for school use.

Sterilisation of Inoculation Loop:

This is done by heating the loop to red heat in a bunsen flame.

To prevent spluttering of droplets from the loop as they heat up, gradually draw the inoculating loop through the flame, heating the handle end first. Allow the loop to become red hot then move whole loop up and down in the flame.

After sterilisation, allow the loop to cool for a few seconds before use. After use, always re-sterilise the loop and allow to cool before putting it down.

Opening culture bottles:

When culture bottles are open there is a risk of contamination from the atmosphere. The following procedure has been devised to minimise contamination.

1. Loosen the cap but do not remove it.
2. Hold bottle in left hand and remove cap with little finger of right hand.
3. Flame neck of bottle by passing neck through a Bunsen or spirit burner flame, backwards and forwards. At this stage something could be introduced or removed from the culture with an inoculating loop.
4. Flame neck again as in stage (3).
5. Replace cap.

The bottle should only be open for the minimum time possible.

Petri dishes:

The plastic dishes obtained from suppliers have been sterilsed by gamma irradiation. The pack should be kept intact as long as possible. On opening the pack, remove only the number of petri dishes you require and reseal the pack by folding over and securing with adhesive tape.

Pouring a plate

The inside of the dish will remain sterile as long as the top remains in position. As there are a few spare dishes in the pack, it is worthwhile using one to practice the technique below.

It will be necessary to pour medium into dishes and to introduce and remove organisms using the inoculating loop. When this is being done, the lid of the petri dish should be lifted off with the left hand and held at an angle over the dish. The operation can then be performed with the right hand.

Do not completely remove the lid. Replace it immediately the operation is complete.

Practice these techniques:

1. Flaming the loop; lifting the petri dish lid; placing the loop inside; removing it; replacing the lid and re-flaming the loop.
2. Holding empty bottle in right hand; removing cap with left hand and placing it on bench; flaming neck; lifting petri dish lid with left hand; pouring from bottle; replacing petri dish lid and replacing cap.

Maintenance of Bacterial Cultures

Bacterial cultures will perform at their very best in all experimental work when they are used as fresh as possible, a culture that is a few days old will perform really well, however when the culture is >7 days old its performance cannot be guaranteed and may even be disappointing.

Cultures are grown up to order, stored in fridge and sent out direct to customers from our lab. If you are unable to use them immediately, but want to use them in the future, then it will be necessary to ensure that they stay viable (able to grow), by maintaining their growth on a regular basis by sub-culturing.

To sub-culture - aseptically transfer a loopful of your culture onto a suitable medium (see below) and then allow it to grow at its optimum temperature until good growth is obtained. The recommended viability times below are what we use here at the Philip Harris Laboratory and therefore are a guide only, but if followed, these cultures can be maintained indefinitely. Their viability can be extended, if stored in a fridge.

Bacteria listed below have very low viability - should be sub-cultured every 1-2 weeks, when stored at room temperature (approx. 21°C)

Lactobacillus bulgaricus	MRS medium	37°C
Lactobacillus casei	MRS medium	37°C

For maintenance purposes, MRS broth can be used, which is easier and quicker to inoculate.

When MRS solid medium is used, growth will be greatly reduced if this prepared medium has been made up for longer than 4-5 months. A lower temperature of 30°C can be used, but growth will be slower.

Bacteria listed below have low viability — should be sub-cultured every 2-3 weeks when stored at room temp.

Acetobacter aceti	Acetobacter medium	30°C
Azotobacter vinelandii	Norris's nitrogen-free medium	25°C
Chromobacterium lividum	Chromobacterium medium	20°C
Photobacterium phosphoreum	Photobacterium medium	25°C
Rhizobium leguminarosum	Yeast Malt medium	25°C
Spirillum serpens	Nutrient agar	30°C
Streptococcus faecalis	Nutrient agar	30°C
Streptococcus lactis	Nutrient agar	30°C
Vibrio sp.	Nutrient agar	30°C
Vibrio natriegens	Vibrio natriegens medium	30°C

Bacteria listed below have good viability - should be sub-cultured monthly, when stored at room temp.

Agrobacterium tumefaciens	Nutrient agar	30°C
Bacillus megaterium	Nutrient agar	30°C
Bacillus stearothermophilus	Nutrient agar	65°C
*Bacillus subtilis**	Nutrient agar	30°C
Erwinia carotovora	Nutrient agar	30°C
Escherichia coli (K12)	Nutrient agar	30°C
Escherichia coli B strain	Nutrient agar	30°C
Micrococcus luteus	Nutrient agar	30°C
Pseudomonas fluorescens	Nutrient agar	30°C
Staphylococcus albus	Nutrient agar	30°C

*(*B.subtilis* becomes translucent with age and can give rise to unpredictable growth, so to ensure success you may prefer to store in fridge and sub-culture on a monthly basis).

Maintenance of Fungal Cultures

Fungal cultures are grown up to order, and sent out direct to the customer. They will stay viable for 1-2 months at room temperature, longer if stored in the fridge.

Yeasts - aseptically transfer a loopful of culture onto Malt medium and grow at 25°C for 3-5 days.

All other fungal cultures — rather than using a loop as with bacterial cultures, it may be easier to use a straight wire that has been fitted to a holder, with the tip of the wire bent at a 90 degree angle. Using this, aseptically cut out a 5mm square piece of fungal culture including 2mm depth of medium, loosen underneath. Pierce this section with the angled wire and transfer to the new medium by inverting it, so that the fungal growth is in direct contact with the recommended medium (see catalogue). Place at 25°C and leave until fully grown, between 3-7 days.

For further information regarding bacterial or fungal cultures, please see current Philip Harris catalogue.

Disposal

It is most important that living cultures or contaminated equipment are always made harmless by sterilisation before being disposed of. Procedures are given below.

Sterilisation by autoclaving

This method relies on steam at 121°C to kill the micro-organisms. If you do not possess an autoclave, a domestic pressure cooker will make a suitable substitute. Contaminated glassware may be autoclaved directly but first loosen caps on screw-topped containers. Plastic materials should be placed in the disposal bags provided. Autoclave the items at 103Kpa (15 psi) for fifteen minutes, after which glassware may be cleaned, washed and re-used. Ideally the disposal bag should be incinerated but otherwise it may be discarded with biological waste.

Sterilisation by soaking or steeping

Chemicals are used to kill the organisms in this method:

1. Use Virkon - A58543 as a 1% w/v solution.
A58555

This is active against viruses, bacteria, fungi and blood contamination.

2. Use a clear phenolic disinfectant.

The use of hypochlorite disinfectants for bacteriology is not normally recommended since they are inactivated by organic material (i.e. agar). In an emergency a freshly prepared 4% hypochlorite solution (sodium chlorate (1)) can be used. The material to be sterilised should be covered with the solution and left for 30 minutes.

Useful Addresses

CLEAPSS — School Science Service (England and Wales)
http://science.cleapss.org.uk/
Tel: 01895 251 496

SSERC — Scottish Schools Equipment Research Centre
www.sserc.org.uk
Tel: 01383 626 070

Supplier details

Philip Harris Education, 2 Gregory Street, Hyde, Cheshire SK14 4RH

Orders and Information:
Tel: 0845 120 4521
Fax: 0800 138 8881

Repairs:
Tel: 0845 120 3211

Technical Support:
E-mail: techsupport@philipharris.co.uk

www.philipharris.co.uk