# Biology/Biotechnology

## Safety in Microbiology

## A Code of Practice for Scottish Schools and Colleges

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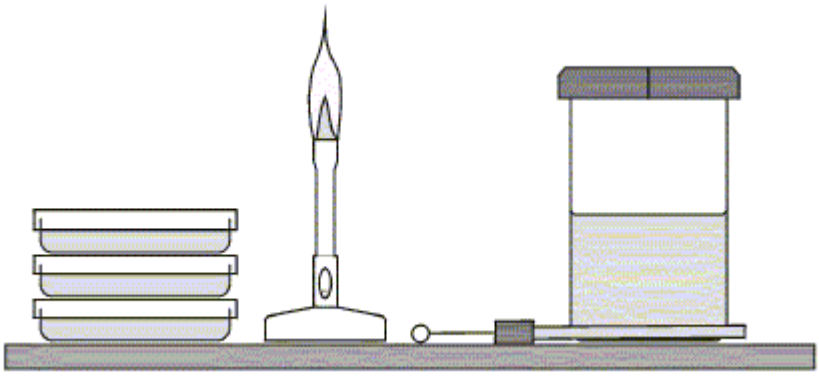
[1] The membership of the original Strathclyde Committees responsible for the 1989 and 1991 editions is detailed in Appendix 7.

# SAFETY IN MICROBIOLOGY



## A Code of Practice for Scottish Schools and for Non-Advanced work in Further Education Colleges

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# INTRODUCTION

**Scope of this code**

The work covered herein spans simple demonstrations and investigations in primary schools and early secondary (for example within science as part of Environmental Studies 5-14) through to Advanced Higher courses taught either in schools or in Colleges of Further Education.

**Central principles**

Practical work involving micro-organisms can have considerable educational value in primary and secondary schools and on into further education. Current curricular changes in science courses, including developments in biotechnology, mean that it is timely to review educational guidance on safety in microbiology.

Without doubt, there are hazards - sources of harm or danger - associated with practical work involving micro-organisms. These include the potential:

- for infection with a disease causing organism (pathogen), or
- for the work to cause an allergic reaction.

Individuals with a reduced resistance to infection may be at particular risk from such hazards. The proportion of such individuals in the population at large would seem to be increasing. This may partly be because diseases causing immuno-deficiencies are now more common and partly because certain medical treatments are more commonly prescribed, including the use of chemotherapy and of immuno-suppressant drugs.

*Hazards*, obviously have to be recognised. Thankfully, the probability of harm being realised in practice is amenable to sensible control. It is this probability of harm, together with the seriousness of any consequence which in practical terms defines degrees of *risk*. Controlling the chances of the realisation of harm in any actual circumstances calls for "preventive" measures. Limiting the consequences is primarily a matter chiefly of planning ahead and putting in place "protective" measures.

Teachers, lecturers and technicians all must be aware of any significant risks in educational microbiological work and of the procedures that are to be followed in order to minimise them. This document describes procedures and protocols which will deliver the educational benefits of microbiological work in ways which then far outweigh any residual risks.

This Code of Practice thus offers a set of preventive and protective measures produced as a result of model risk assessments. As such it can be adopted so as to meet the requirements of the COSHH Regulations and of other related health and safety legislation.

Central to this strategy is the concept of **levels of work**, whereby practical work for a particular level is determined by a combination of **risk factors** which include the:

cont./over

- choice of micro-organisms
- growth media used
- choice of a suitable inoculum
- incubation conditions
- scale of operations
- handling operations permitted
- degree of containment
- teacher training
- availability of trained support staff
- age of the pupils or students and
- available resources

There are three levels of work. In general, these levels are related to the educational stage of pupils and students. Thus in primary school only level 1 work may be attempted; in secondary schools, pupils in S1 to S4 will normally be restricted to work at level 1 or level 2, whilst for some post 16 national courses students may, in addition, participate in level 3 work. Technicians or, in some cases, teachers who may prepare materials for level 2 work may have to perform level 3 tasks. They should be trained, accordingly - to level 3.

## *Bioreactors (fermenters)*

Use of bioreactors increases the potential risks involved in microbiology, since larger volumes of liquid culture medium are used (see the risk factor- "scale"). For this reason, the choice of organisms and procedures which are permitted for use in bioreactors is more limited than that for small scale operations. Here, the term bioreactor, is used to refer to vessels other than Petri dishes or McCartney bottles and Universal containers in which micro-organisms are grown in liquid medium. This includes a wide range of vessels from the very simple to growth systems with elaborate sensing and control devices.

The purposes of using bioreactors in teaching include the following applications.

Applications which demonstrate:

- the use of growth vessels which simulate those used in commercial biotechnology and
- the culturing of an organism for biomass production.

Applications which allow studies of:

- the effect of various factors on growth of the organism e.g. temperature, pH, aeration;
- production of a product e.g. ethanol, specific proteins;
- where appropriate, datalogging and process control;
- batch, 'fed-batch' and continuous fermentation processes and
- the activity of enzymes or cells in an immobilised state or in an aqueous state.

Given sensible precautions, the educational value of these activities far outweighs the small degree of risk then involved.

## 1. GENERAL SAFETY CONSIDERATIONS

The general principles and considerations upon which this document is based are outlined below.

1.1 All microbiological materials, cultures, media, environmental samples etc from whatever source should be treated as though they were a potential source of pathogens.

1.2 The chance of contamination of the medium in which micro-organisms are growing and/or of micro-organisms escaping from the medium to the environment must be minimised.

1.3 Only a limited range of species and strains of micro-organisms may be used for each level of work (see appendices 1 to 4).

1.4 The use of growth media that may encourage the growth of pathogens is generally prohibited.

1.5 Sources of environmental samples for study are restricted.

1.6 The conditions under which micro-organisms may be grown are restricted.

1.7 The handling operations which may be undertaken with micro-organisms are limited.

1.8 The level of work with micro-organisms that a teacher or lecturer may undertake will be limited by the training that the teacher or lecturer has undergone.

1.9 The level of work with micro-organisms that may be undertaken in an educational establishment will be limited by the resources at hand and by the availability of trained support staff in that establishment.

1.10 As a result of the limits which are placed on work at level 1 and level 2, protective clothing is not essential for work at these levels. Suitable protective clothing (e.g. lab coat) is required for level 3 work.

1.11 Preparation, sterilisation and disposal of microbiological materials should not be carried out by students other than as part of a supervised and structured learning activity.

1.12 If teachers or lecturers require to use micro-organisms outwith those named in this document, or to depart from the procedures herein, then a separate risk assessment of the proposed procedures must be carried out from first principles. *Risk Assessment form B for Processes Involving Micro-organisms* from *Preparing COSHH Risk Assessments for Project Work in Schools*, SSERC, 1991 (revision pending) is suitable for recording the results of such a risk assessment.

## 2. THE LEVELS OF WORK

It is appropriate to define three levels of working with micro-organisms, which might be encountered in schools and further education, according to the risks which they present and the type of training required before teachers or lecturers should embark upon them.

(*Note that these are not the same as the 'levels of containment' as used by professional microbiologists*).

### Work at Level 1

The only micro-organisms permitted for use at this level are those which carry little, if any, known risk and which may be used in experiments by teachers with no specialist training. These can be disposed of safely using good domestic practices. Faulty technique may prevent educational ends being met, or it lead to the demise of the micro-organisms used. It is unlikely, however, to place pupils or staff at any significant risk.

2.1 Only micro-organisms from Appendix 1 may be used.

2.2 No specialist training is required.

2.3 No specialised facilities or equipment are required.

2.4 Normal good domestic hygiene measures must be used.

2.5 The use of living micro-organisms in the preparation of food to be eaten must **not** be carried out in classrooms or laboratories. Such preparations (of e.g. yoghurt) must be carried out only in rooms where food is normally prepared or where suitable clean and hygenic work surfaces can be temporarily arranged.

2.6 The natural spoilage of plant material (e.g. fruit) may be observed provided the material is kept in a loosely stoppered container which can be disposed of unopened in a refuse bin.

2.7 The spoilage of animal material (e.g. meat, meat products) must **not** be studied.

## Work at Level 2

Work at level 2 can involve a wider range of recommended micro-organisms and inoculation techniques and involves the use of sterile media and sterile equipment.

2.8 **For small scale** microbiological work at level 2 (e.g. using Petri dishes and/or McCartney bottles or universal containers) only micro-organisms named in Appendix 2 can be used.

2.9 Where **larger volumes** of culture medium are used (e.g. in a bioreactor [fermenter]) then **only** micro-organisms with "unusual" growth requirements such as those requiring high salt or acid conditions can generally be used. This allows for growth of the intended micro-organism at the expense of undesirable contaminants.

Appendix 4 gives the names of permitted micro-organisms and their special growth conditions including the temperature range for use with fermenters.

2.10 Normal laboratory facilities (including hand washing) and specialised facilities for the preparation of media and disposal of cultures are required. (See section 3 on resources).

2.11 Autoclaving is the preferred method of sterilisation of equipment. Only where it is impractical to autoclave (e.g. due to the properties of the materials used in construction of the equipment or because of its size) may chemical disinfection be used.

2.12 Protective clothing is not essential.

2.13 All sterilisation required for level 2 work is a **level 3 task** (see **2.22**)

2.14 Transfer of prepared sterile medium to sterile culture vessels is permitted.

2.15 Samples taken from solid cultures of Appendix 2 micro-organisms can be used to inoculate sterile solid or sterile liquid medium.

2.16 Samples of Appendix 1 and Appendix 4 micro-organisms taken from liquid cultures by inoculating loop can be used to inoculate sterile solid medium or sterile liquid medium in small scale culture. At level 2, students may also inoculate sugar solutions with yeast cells for large scale liquid culture work.

2.17 Removal of liquid samples from a fermenter, with the exception of yeast and sugar solutions, must only be carried out by a person trained to perform **level 3 tasks**.

2.18 Samples from carefully chosen areas of the environment may be used, but **only to inoculate sterile solid media**.

In particular, **samples must not be taken for culture from**:

*a) human* or other animal body surfaces;*
*b) body fluids and secretions;*
*c) animal cages or aquaria;*
*d) lavatories;*
*e) faecal material;*
*f) poultry, eggs or areas which have been in contact with poultry;*
*g) meat or meat products;*
*h) dead animals;*
*i) milk which has **not** been pasteurised;*
*j) soft, unpasteurised, cheeses;*
*k) water sources likely to contain faecal or sewage pollution;*
*l) soil fertilised by animal manure or fouled by animal faeces;*
*m) mud (e.g. from a pond or field).*

** The classic 'finger dabs' experiments on washed and unwashed human hands was effectively ruled out by the first edition of this code. Re-assessment of the risks from this practical has led to its reinstatement here, provided that only sterile plates are used and they remain closed after inoculation and through to disposal. Similarly 'handshaking' experiments which demonstrate the passing on of microbial contamination may be performed so long as only the coloured organism suggested in Appendix 2 for this purpose is used.*

2.19 All cultures, irrespective of source, must be kept closed during incubation and subsequent examination.

2.20 Teachers of level 2 work must be trained in dealing with spillages.

2.21 In order that teaching at level 2 may be safely carried out, personnel trained at level 3 must be available for the supervision and performance of those level 3 tasks that are relevant to the level 2 work.

## Work at Level 3

2.22 The following level 3 tasks are normally required to be carried out in an establishment in support of level 2 work:

*a) order, receipt, labelling and storage of cultures;*
*b) preparation of sterile media and sterile equipment;*
*c) sub-culturing;*
*d) sampling from bioreactors*
*e) sterilisation and disposal of cultures;*
*f) sterilisation of used equipment;*
*g) management of incidents of spillage;*
*h) staining of incubated plates (e.g. starch agar[1]).*

Work at level 3 can involve additional micro-organisms and a wider range of inoculation and transfer techniques (cont./over).

Footnote 1 (bullet point *h*): Where these have been inoculated with an organism. Uninoculated starch agar plates used for enzyme specificity plates may be opened and stained by students. To prevent contamination, uninoculated starch agar plates should be disposed of with due care and as soon as may be sensible after use. Autoclaving, where practicable, is the preferred method.

2.23 For small scale microbiological work at level 3, micro-organisms from Appendices 1 to 4, inclusive, may be used.

2.24 For larger scale microbiological work (e.g. in a bioreactor) only those organisms from Appendix 4 grown under their special growth conditions may be used.

2.25 Suitable protective clothing (e.g. lab coat) must be worn.

2.26 An autoclave may be used by students under the supervision of a teacher or technician trained at level 3, **but not on a routine basis.**

2.27 Samples taken from solid cultures of micro-organisms from Appendices 1 to 4 can be used to inoculate sterile solid or sterile liquid medium.

2.28 Samples taken from liquid cultures of micro-organisms from Appendices 1 to 4 can be used to inoculate sterile solid or sterile liquid medium.

2.29 Samples may be removed from bioreactors under the supervision of a teacher or technician trained at level 3. Because of the risks from contaminants, samples must not be removed after the culture has ceased active growth nor if the specified culture conditions have changed. Exceptions to this specific requirement would include:

- growth curve investigations which have to be continued beyond the 'log' phase to establish the complete *'S'* shaped growth curve and
- investigations of *diauxic* growth with different substrates.

# 3. RESOURCES

Before safe microbiological practice may be reasonably assured, certain resources are required for each level of work.

**Resources required for Level 1 Work**

3.1 Resources for good domestic hygiene are required.

3.2 Hand washing facilities, including a sink, soap, water, paper towels and a bin should be readily accessible.

3.3 A household disinfectant, suitable for wiping work surfaces, must be available.

3.4 A large, transparent container, which can be loosely stoppered, is most suitable for studying the spoilage of plant material.

3.5 A sink is required for the disposal of liquid waste.

3.6 Access to normal solid refuse disposal facilities is required for the safe disposal of containers in which the natural spoilage of plant materials has been studied.

**Resources required for Level 2 and level 3 Work**

3.7 Normal laboratory facilities are essential. These include work surfaces, a sink and a gas supply.

Absorbent, or otherwise unsound, work-surfaces should not be used. If absorbent surfaces are covered with a non-absorbent material (e.g. waterproof backed bench covering), this would be acceptable. Varnished wooden surfaces in good condition are acceptable. Temporary work surfaces such as a set of separate, portable, laminated boards, are also acceptable.

Handwashing facilities, including sink, soap, water, paper towels and a bin must be available. Suitable protective clothing (e.g. lab coat) is required for level 3 work.

3.8 Preparing work surfaces. For much work at level 2 it suffices that work surfaces are cleaned with hot water and detergent (or a surfactant disinfectant) prior to commencing work. Freshly diluted 1% hypochlorite solutions (a good quality commercial bleach e.g. *Domestos*, *Chloros* or laboratory sodium chlorate(I) [hypochlorite]) may also be used to disinfect non-absorbent surfaces but care must then be taken to avoid contamination of the skin or clothes. *Virkon* is a suitable substitute but is relatively expensive. Where *Virkon* is used in discard jars (see below), it is good practice to use a different disinfectant for swabbing work surfaces.

3.9 Any discard jars, used for contaminated used equipment, must contain freshly diluted clear phenolic disinfectants such as *Stericol* or *Hycolin* at 2% v:v or *Virkon* at a concentration of 1% v:v.

**Note that the manufacture of clear phenolics is being discontinued.**
Schools should use Virkon once their supplies of clear phenolic have been used up. When in solution, virkon has a pink colour which gradually fades to colourless. Activity of a fresh solution diminishes by 10% after 7 days. It should not be used once the pink colour has faded. Virkon action is based on a multi-component oxidising system but it does not contain any chlorine.

3.10 Disinfectants which contain available chlorine [eg "Chloros", sodium chlorate (1) (hypochlorite) or bleach] may be used in suitable dilution to surface sterilise plant material (eg beans, peas).

3.11 Clear phenolic disinfectants (see 3.9) or Virkon must be available for treating spillages and for adding to bioreactors after use.

3.12 Petri dishes should normally be of the disposable plastic type. If glass Petri dishes are used they must be sterilised for re-use by autoclaving or with dry heat. Where glass Petri dishes are re-used chemical disinfection is not acceptable for the purposes of sterilisation.

3.13 McCartney and, or, universal bottles should be made of glass, with screw-top lids.

3.14 Where metal inoculation loops are used, they must be of the following design:

24 SWG nichrome wire should be bent around a match stick, making sure that the loop formed is fully closed. The overall length of the wire, including the loop, should be no more than 50 mm. Loops must be attached to metal "chuck" type holders.

(Loops conforming to the above design may be made, or they can be purchased from scientific suppliers.)

3.15 Proprietary cotton buds may be used as inoculation swabs to sample the environment.

3.16 Steam sterilisation is best carried out using a laboratory autoclave. A pressure cooker of the domestic type is also suitable but is better reserved for small-scale work.

3.17 Glass flasks (suitably thick-walled), demijohns or similar containers can be used as simple bioreactor vessels.

3.18 Reactor vessels must be constructed from robust material.

3.19 The design of bioreactor vessels should be such as to permit ease of cleaning.

3.20 The shape of bioreactor vessels should give stability in use. If necessary, the vessel should also be supported.

3.21 A tray which is large enough to contain any spillage from a bioreactor vessel is required. If possible, the tray should allow drainage into a sink or carbuoy.

3.22 Personnel trained for level 3 work are required in the establishment to allow level 2 work to be carried out.

**Resources required for Level 3 Work**

3.23 Personnel trained at level 3 must have access to the following additional resources, in order to prepare for, and support, level 2 work:

a) a hard non-absorbent work surface (e.g. plastic laminate), which is away from direct sources of draughts;

b) suitable protective clothing (e.g. lab coat);

c) an autoclave;

d) a spillage kit containing:

- *a screw-capped bottle containing a measured quantity of undiluted disinfectant with the correct volume of water needed to dilute it marked on the side*
- *a quantity of paper towels*
- *a pair of autoclavable tongs or a small plastic dustpan*
- *a pair of disposable plastic gloves*
- *an autoclavable waste disposal bag*
- *an autoclavable container, with suitable means of closure into which contaminated, broken glass may be easily transferred for sterilisation.*

The spillage kit should be placed in a prominent site for ease and speed of access.

## 4. GOOD PRACTICE IN USING MICRO-ORGANISMS

### General

4.1 The 'precautionary' practice, of treating all microbiological materials as potential sources of harmful contaminants, forms the basis of good laboratory practice in microbiology.

4.2 Techniques for handling microbiological materials are described in *Microbiological Techniques* published by HSDU and SSERC 2001. (An interactive version on CD ROM is also available [as from Summer 2002]).

4.3 When bioreactors are used for enzyme experiments, the substrate, product and/or the enzyme could be a suitable source of nutrients for the growth of micro-organisms. Therefore, the safety considerations which apply to the use of bioreactors with micro-organisms may be applicable also to enzyme experiments, particularly if carried out over extended periods, i.e. more than four hours.

4.4 All hand-to-mouth operations, including eating, chewing, drinking, smoking, sucking pens and pencils and licking labels are forbidden in any laboratory used for microbiology. The consumption of any product from a bioreactor is not permitted.

4.5 Everyone working with micro-organisms must wash their hands thoroughly, using soap and water, both before and after microbiology work.

4.6 Exposed cuts should be covered with waterproof dressings.

4.7 Long hair should be tied back.

4.8 Refrigerators or cupboards used for storage of micro-organisms and micro-biological material must not be used for storage of food or drink.

### Good Practice at Level 1

4.9 Work surfaces must be cleaned using hot water and detergent or a household disinfectant, prior to starting work and after completion of the work.

4.10 Only micro-organisms named in Appendix 1 can be used for work at level 1. These micro-organisms must be purchased from an approved supplier or culture collection.

4.11 Containers used for microbiology work must be clean.

4.12 Appendix 1 micro-organisms should be grown in an appropriate medium (e.g. sugar solution for yeast, UHT milk for yoghurt). Suppliers' instructions should be followed, where applicable.

4.13 The growth of micro-organisms should be carried out in covered containers (e.g. with aluminium foil covers or non-absorbent cotton wool plugs).

4.14 The growth of micro-organisms should normally be carried out at room temperature and, except for yoghurt making, never above 30°C.

4.15 The growth of micro-organisms should be continued for the minimum time required to show the desired effect (usually no longer than 24-36 hours for bacteria and several days - possibly 1 week - for fungi).

4.16 Microscope slides, prepared using micro-organisms from Appendix 1, can be safely disposed of without being sterilised.

4.17 Liquid cultures should be poured down the sink and washed away with plenty of water.

Solid cultures (e.g. yeast dough) can be disposed of in the bin used for solid, domestic type, waste.

4.18 Containers should either be cleaned immediately after use or disposed of in the bin for solid refuse.

4.19 The material chosen for the study of the spoilage of plants should be placed in a large, clean, dry, loosely-stoppered container. The container should be tightly stoppered before the contents are studied by pupils and the container disposed of unopened in the bin for solid refuse.

**Good Practice at Level 2**

4.20 Working surfaces should be wiped with hot water and detergent, or a surfactant disinfectant, before and after use.

**Cultures**

4.21 Where micro-organisms from Appendices 1, 2 or 4 are used, they must be purchased from an approved supplier.

4.22 Cultures should be purchased specifically as and when required. Storage of cultures should be for the minimum time practicable.

4.23 Freeze dried cultures may be used but only where conventional slopes or other more easily handled forms are not offered by the approved supplier. The person opening the ampoules should be trained in the simple techniques necessary for this to be performed safely and without consequent contamination.

4.24 Purchased cultures should be dated on arrival and placed in a closed container in a refrigerator or cupboard, both of which should be labelled with Biohazard labels.

4.25 A log must be kept of all cultures showing:

*a) name of micro-organism;*
*b) supplier;*
*c) date of receipt;*
*d) number of sub-cultures made;*

*e) date of each sub-culture;*
*f) by whom sub-cultures were taken;*
*g) date of disposal.*

4.26 All sub-culturing for level 2 work must be carried out by a person trained for level 3 work.

4.27 All cultures should be sterilised and disposed of by a person trained for level 3 work as soon as is practicable after use. At the end of the teaching programme all cultures (including the original stock cultures) should normally be sterilised and safely disposed of.

4.28 Under no circumstances should any culture be kept for longer than one year.

4.29 Only sterile milk inoculated with dried yoghurt bacteria may be re-opened (e.g. to measure pH). Milk may only be inoculated with dried yoghurt bacteria: *Lactobacillus bulgaricus, Lactobacillus lactis* or *Strepococcus lactis.*

4.30 Where possible, fungi that produce large numbers of air borne spores should be handled before sporulation occurs. This is particularly important for some species, e.g. those of *Aspergillus* and *Penicillium* which produce large numbers of easily dispersed spores and to which serious allergic reactions can occur.

**Media**

4.31 Enriched, or selective, media which may encourage the growth of pathogens must not be used. This includes media such as blood agar, bile salts, McConkey's agar, dung or faecal agar and corresponding broths or media which use animal sera or blood.

4.32 Antibiotics should not normally be incorporated into growth media and only commercially produced paper impregnated antibiotic discs should be used. (But, see note in Appendix 3 on antibiotics and certain protocols at level 3).

4.33 The volume of culture medium should be **no larger** than is necessary to carry out the investigation.

4.34 Media for use in Petri dishes should be cooled in a thermostatically controlled water bath at **55$^{o}$C** before pouring. The necks of media containers should be flamed before pouring.

4.35 When introducing sterile media from a McCartney bottle or universal container into an empty sterile Petri dish, the following procedure should be observed:

*a) the Petri dish should be placed lid uppermost on the bench;*
*b) the lid should be opened just enough to allow the operation and must be held in the hand, not placed on the bench;*
*c) the lid must be opened for the minimum amount of time;*
*d) the mouth of the McCartney bottle or universal container must be flamed when the lid is removed, and - unless emptied - flamed again before the lid is replaced.*

4.36 Prior to use agar plates must be inspected and contaminated plates discarded, sterilised and disposed of. The surface of the agar must be dry.

4.37 Plates with excessive condensation on the lids should be discarded.

## Inoculation

4.38 Containers prepared for inoculation should be labelled with a self-adhesive label, or using a waterproof marker, stating:

*a) date of inoculation;*
*b) nature of the inoculum;*
*c) name of operator;*
*d) nature of media.*

4.39 Inoculating loops should be sterilised before being introduced into a culture. The entire length of the wire and loop, should be heated to red heat in a blue bunsen flame and allowed to cool, without coming into contact with any surface. The inoculation loop should be sterilised after use.

4.40 The mouths of culture bottles should be flamed when lids are removed and flamed again before their replacement. Lids should not be placed on the bench. With practice it is possible to manipulate bottles, lids and loops without any of them leaving the hands. Should a lid fall to the bench or floor, it should be replaced and the bottle sterilised and contents safely disposed of.

4.41 Cotton buds must only be used for sampling from the environment and must not be used for culture transfer work. Cotton buds must only be used as swabs for inoculating agar media in Petri dishes. Used cotton bud swabs should be held in a discard jar prior to their safe disposal.

4.42 Petri dish lids should be opened for the minimum amount of time necessary to complete the inoculation procedure.

4.43 Once inoculated, the lids of McCartney bottles or universal containers should be tightened.

4.44 Once inoculated, each Petri dish must be sealed diametrically, using transparent adhesive tape and incubated base uppermost.

4.45 When introducing antibiotic discs to freshly inoculated Petri dishes, the following procedure should be observed:

*a) only commercially available paper impregnated antibiotic discs may be used;*
*b) the Petri dish should be placed lid uppermost on the bench;*
*c) the lid should be opened just enough to allow the operation and must be held in the hand, and not placed on the bench;*
*d) the lid must be opened for the minimum amount of time;*
*e) antibiotic discs must be transferred to the Petri dish with sterile forceps;*
*f) after the transfer operation, the forceps must be flamed or placed in a discard jar;*
*g) the Petri dish should be taped and thereafter must never be re-opened*
*h) on completion of observations, the Petri dish must be autoclaved and disposed of.*

**Incubation**

4.46 In case of spillage, bottles containing inoculated liquid medium should be placed in a secondary container during incubation.

In experiments which involve production of biogas (e.g. methane), there is a potential for build-up of flammable gases. Therefore, eye protection must be worn, and a safety screen should be used.

4.47 Areas of restricted access to students must be used for all incubations.

4.48 Incubations are not normally to be carried out above 30$^{o}$C; exceptions to this are given in Appendices 3 and 4.

4.49 No incubation should be continued for a longer time than is essential to obtain the required result.

4.50 After incubation, containers should not be re-opened.

## Bioreactors (fermenters)

4.51 Bioreactor vessels should be inoculated with an actively growing inoculum to reduce the risk of a contaminant becoming established. This inoculum should be a significant fraction (e.g. 10-20 per cent) of the total volume of the medium.

4.52 Experiments to demonstrate biogas production should only use plant substrates (e.g. crushed beans) and must have no other added inoculum or enrichment media.

4.53 Electrical equipment should be sited at a safe distance from the bioreactor vessel and wet working areas whenever possible. Care should be taken to keep bioreactor mains, air and water feed lines tidy.

4.54 Mains powered electrical apparatus used with a bioreactor system must be of commercial design, intended for school use and supplied by a reputable supplier. All such mains apparatus should be protected by a safety 'cut out', i.e. an appropriate residual current circuit breaker. All other apparatus should operate from a power pack with a maximum output of 25 volts.

4.55 The bioreactor system should be located to avoid the possibility of accidental or deliberate interference.

4.56 The bioreactor must be set in a tray which is large enough to contain any spillage. If possible, the tray should drain into a sink or carboy.

4.57 The bioreactor vessel lid (where applicable) must have an airtight fit and should be secured, to prevent accidental opening of the vessel.

4.58 Ports holding probes or other inserts in bioreactors must be effectively sealed.

4.59 Unused bioreactor ports must be effectively stoppered.

4.60 Where a rotating stirrer bar or impeller is fitted to a bioreactor, all probes and inserts should be carefully positioned so as to avoid mechanical damage from such agitation devices.

4.61 The bioreactor vessel must be adequately vented to prevent the build up of pressure. A wine-making trap or substantial non-absorbent cotton wool plug should be sufficient to trap any fine spray in a simple bioreactor (e.g. a flask or demijohn).

4.62 Where bioreactors have air inlet lines, these must be fitted with an in-line filter.

A bacteriological filter or glass tubing packed with 75 mm of non-absorbent cotton wool is recommended. Filters must be sterilised before and after use. Filters should be replaced after each use, or in accordance with manufacturers' instructions.

Any exit air lines or vents must also be fitted with an in-line filter, as specified above. Such exit air lines or vents must be positioned vertically above the culture vessel, to prevent condensation blocking the air-line or filter.

Attention must be given also to the possibility of siphoning-back in air-lines. They must be so positioned as to avoid the possibility of a siphon or be fitted with a non-return valve.

4.63 In bioreactors with high aeration levels, or temperatures over 30$^{o}$C, excessive water loss can be overcome by fitting a condenser (vertically) between the growth vessel and the exit air filter.

4.64 Excessive foam formation in bioreactors must be avoided. It may lead to the blocking of the exit air line (air vent) and cause build-up of excess pressure. Foaming can be avoided by the addition of one drop of silicone anti-foaming agent.

### Immobilised Cells

4.65 For experiments with immobilised cells, micro-organisms must only be selected from Appendix 1.

### Disposal

4.66 Microscope slides, prepared using micro-organisms from Appendix 2, must be placed in a discard jar, with an appropriate disinfectant, before being disposed of.

4.67 Arrangements should be made for the return of all microbiological materials for sterilisation and disposal.

### Spillages

4.68 If a gross spillage occurs (e.g. from a bioreactor vessel), the room must be cleared immediately and no one should re-enter the room for thirty minutes. A person trained for level 3 work must be informed immediately.

4.69 Small scale spillages (e.g. from McCartney bottles or universal containers, or breakages of Petri dishes) must be covered with paper towels soaked with a clear phenolic disinfectant for at least ten minutes before being cleared away by a person trained for level 3 work. Care should be taken to avoid inhalation of any aerosol cloud formed by the spillage. Spillages on skin and clothing must be washed with soap and water.

## Level 3 Tasks Required to Support Level 2 Work

### Sterilisation

4.70 All vessels and other apparatus must be sterilised before and after use.

Sterile media and equipment should be prepared by autoclaving at **121°C for 15 minutes** (thus giving a pressure of 103 $kNm^{-2}$ or 15 $lbf\ in^{-2}$ steam pressure) or at **126 °C for 10 minutes**. No single container of liquid medium should contain more than $500cm^3$ unless the holding time is suitably prolonged and sterility is checked. A *Browne's* tube or other time/temperature indicating device, with an appropriate range, should be included in the load when an autoclave is brought into use after a period in storage, and occasionally thereafter. (An autoclave is a designated *control measure* in terms of COSHH requirements and its efficacy must be checked at sensible intervals).

Media for use in fermenters should be sterilised by use of an autoclave, as above, or by following manufacturers' instructions.

4.71 Items of equipment which cannot be steam sterilised can be decontaminated by the use of chemical disinfectants, e.g. a clear phenolic or Virkon.

4.72 Glassware which cannot be conveniently autoclaved may be sterilised by dry heating in an oven maintained at 160°C for two hours.

## Sub Culturing

4.73 Sub-culturing should be carried out only for immediate requirements and kept to the minimum necessary for level 2 work to take place.

4.74 Freshly inoculated Petri dishes, for use with antibiotic discs, should be prepared by using a sterile pipette or sterile dropper to transfer a few drops of a liquid culture of organisms from Appendix 2 to a sterile agar plate. The inoculum should be spread using a sterile spreader (lawn plate).

## Disposal

4.75 All microbiological materials should be sterilised by autoclaving before disposal.

4.76 Sterilised liquid waste should be flushed away with a large volume of water. Glassware should then be washed out immediately.

4.77 Used Petri dishes should be autoclaved in disposable autoclavable bags which are loosely tied. Only after autoclaving should these bags then be sealed and placed in a refuse bag before disposal in a refuse bin. It is critical that Petri dishes are loosely packed in the bags and that the autoclave as a whole is not overloaded. If these requirements are not met, then air may not be properly displaced and sterilisation cannot be ensured.

## Spillages

4.78 Spillages should be treated as follows:

*a) Action as detailed in 4.68 and 4.69.*

*b) A lab coat and disposable gloves must be worn.*

*c) The debris should be picked up with autoclavable tongs and put into an autoclavable bag or swept into a plastic dustpan using paper towels.*

*d) All contaminated debris and paper towels must be autoclaved before disposal. The tongs or dustpan must be autoclaved or covered with clear phenolic disinfectant, or a solution of Virkon, for twenty-four hours.*

## Additional Requirements for Good Practice at Level 3

### Cultures

4.79 Where micro-organisms from appendices 1, 2, 3, or 4 are used, they must be purchased from an approved supplier or recognised culture collection.

4.80 All subculturing for level 3 work must be carried out by a person trained for level 3 work. (Note that post-16 students may be trained to carry out a range of level 3 tasks).

### Inoculation

4.81 When using pipette transfer to inoculate Petri dishes the following procedure should be observed.

*a) Sterile disposable plastic pipettes may be used.*

*b) Glass pipettes should have a non-absorbent cotton wool plug inserted in the wide end before being sterilised in a closed container.*

*c) Material should be drawn in and expelled from the pipette by means of a bulb or similar device. Mouth pipetting must not be carried out.*

*d) Care must be taken to avoid aerosol formation (e.g. by not generating bubbles).*

*e) Used pipettes must be placed into a container of clear phenolic disinfectant or Virkon. Any contaminated bulbs should be disinfected and then sterilised by autoclaving. Glass pipettes also may then be autoclaved and cleaned before storing. They must be autoclaved again before re-use. Disinfected disposable plastic pipettes, however, must not then be re-used for any purpose.*

*f) Fluids into which serial dilutions are made must be sterile.*

*g) Where liquid inoculum is to be spread over plates of solid medium, any spreader to be used must be sterile (an alcohol flamed, glass spreader or of a sterile disposable type).*

### *Incubation*

4.82 Cultures inoculated and incubated on solid media may be opened by a student only after the following conditions have been met:

a) The solid medium was inoculated from a culture (or a mixed culture) of micro-organisms from appendices 1, 2, 3 or 4, prepared by a person trained for level 3 work.

b) The incubated cultures have first been visually checked for contamination by a person trained for level 3 work.

**Bioreactors (fermenters)**

4.83 A bioreactor may be set up, sterilised as appropriate before use and inoculated with an organism from Appendix 4 by a student under the supervision of a teacher, lecturer or technician trained to level 3.

**Spillages**

4.84 Gross spillages must not be dealt with by students (4.68, 4.69). Students should be trained to deal with small-scale spillages and may also experience, through simulation, the problems associated with tackling incidents on a larger scale.

**Sterilisation**

4.85 Sterile media may be prepared by a student under the supervision of a teacher, lecturer or technician trained for work at level 3, **but not on a routine basis**.

**Disposal**

4.86 Cultures may be autoclaved and disposed of by a student under the supervision of a teacher, lecturer or technician trained for work at level 3, **but not on a routine basis.**

4.87 The chemical disinfection, and disposal, of bioreactor contents must be carried out only by a person trained for work at level 3.

## APPENDICES

### Selected micro-organisms for Scottish Courses

The micro-organisms listed in Appendices 1, 2, 3 (and 4) have been selected in order to give adequate coverage of current course work in Scotland. These organisms present minimal risks, given good practice. The organisms listed have been selected from fuller, revised, listings endorsed by a range of relevant organisations and first published by ASE. These fuller lists were also re-published in SSERC Bulletin 194, Summer 1998. The fuller listings also give points of educational use or interest and comment on the ease with which organisms can be cultured and maintained. Further enhancement is planned for these tabulated listings so as to include practical culturing tips and other useful information.

**It is important to note that it is not intended that the selected organisms in Appendices 1-4 should be considered as definitive, nor complete, lists.**

Further development of courses and projects, particularly at levels 2 and 3, may well require the use of micro-organisms which do not currently appear on the lists. Therefore it is recognised that regular review of the recommended lists of micro-organisms is necessary to take account of changes in course needs and in the safety literature.

All micro-organisms named in Appendices 1-4 must be purchased from an approved supplier or obtained from a recognised UK culture collection (usually, but not always, more expensive). If teachers require to use micro-organisms outwith those named in this document, or to depart from the approved procedures, then that is by no means ruled out. A risk assessment from first principles, however, must first be carried out. *Risk Assessment form B for Processes Involving Micro-organisms* from *Preparing COSHH Risk Assessments for Project Work in Schools*, SSERC, 1991 (under review) is suitable for recording the results of such a risk assessment.

A less demanding, initial, approach is simply to modify a published protocol so that it complies with this code. It will then be covered by default since it will take into account the results of a 'general' or 'model' risk assessment.

**APPENDIX 1**

## Selected Organisms for Work at Level 1

Breadmaking yeast (*Saccharomyces cerevisiae*)

Dried yoghurt cultures (bacteria used to make yoghurt)

Blue-green algae

Green algae

Free living protozoa

Lichens

Slime moulds

## APPENDIX 2

### Selected Organisms for Work at Level 2

All micro-organisms listed in Appendix 1 and, in addition, the following organisms:

### Bacteria

***Acetobacter aceti***
*Agrobacterium tumefaciens*
*Azotobacter species*
*Bacillus megaterium*
*Bacillus stearothermophilus*
*Bacillus subtilis*
*Cellulomonas* species
*Chromatium* species
***Janthinobacterium lividum***
(also called *Chromobacterium lividum*)
*Erwinia caratovora*
(also called *E. atrospetica*)
*Gluconobacter oxydans*
*Lactobacillus species*
*Micrococcus luteus*
(also called *Sarcina lutea*)
*Photobacterium phosphoreum*
*Rhizobium species*
*Rhodopseudomonas palustris*
*Spirillum serpens*
*Staphlococcus epidermidis*
*Streptococcus lactis*
*Streptococcus thermophilus*
*Vibrio natriegens*
(also called *Beneckea natriegens*)

***Viruses***
*Cucumber Mosaic Virus*
*Potato Virus X*
*Potato Virus Y* (not the virulent strain)
*Tobacco Mosaic Virus*
*Turnip Mosaic Virus*

### *Fungi*

**Agaricus bisporus**
*Armillaria mellea*
*Aspergillus oryzae* (strain CMI 044242) *
*Botrytis cinerea*
*Botrytis fabae*
*Chaetomium globosum*
*Coprinus lagopus*
*Fusarium graminearum*
*Fusarium solani*
*Fusarium oxysporum*
*Helminthosporium avenae*
*Kluveromyces lactis*
*Mucor hiemalis*
*Mucor mucedo*
*Myrothecium verrucaria*
*Neurospora crassa*
*Phaffia rhodozyma (e.g. coloured organism)*
*Penicillium expansum*
*Penicillium roquefortii*
*Physalospora obtusata*
*Phycomyces blakesleanus*
*Phytophthora infestans*
*Pleurotus ostearus*
*Pythium de baryanum*
*Rhizopus oligosporus*
*Rhizopus sexualis*
*Rhizopus stolonifer*
*Rhytisma acerinum*
*Saccharomyces cerevisiae*
*Saccharomyces diastaticus*
*Saccharomyces ellipsoides*
*Saprolegnia litoralis*
*Schizosaccharomyces pombe*
*Sclerotinia fructigena*
*Sordaria fimicola*
*Sporobolomyces species*
*Trichoderma reesei*

(* *CMI = Commonwealth Mycological Institute)*

**APPENDIX 3**

**Selected Micro-organisms for Work at Level 3**

For work at level 3, only micro-organisms listed in Appendices 1, 2 (and also 4) together with those listed below may be used:

**Bacteria** ***E. coli* strain NCTC 10537**
(also referred to as **E. coli strain B**, 'phage host for T 'phages, T1-T7, and λ 'phage).

***E. coli* strain NCTC 10538**
(also referred to as ***E. coli* K12 strain**).

Some *E. coli* strains are associated with health hazards. Therefore **only** strains B and K12 detailed above may be used. The two strains of *E. coli* specified should be suitable for all projects which require the use of *E. coli*.

**Viruses** **Bacteriophage** (T type host is ***E. coli***).

## Note on incubation temperatures and antibiotics:

Some recently developed biotechnology kits have transformation protocols using the pGLO gene set for the expression of fluorescent green protein in debilitated strains of *E. coli.*

a) These protocols may call for incubation at 37° C. Since the strains in question are highly debilitated, and unlikely to survive outwith the laboratory, this use of 37° C is accepted in this specific context. In practice, however, it should normally be possible to so plan the timing of such work as to make feasible the use of somewhat lower temperature (e.g. 30° C).

b) The protocols also require incorporation of antibiotic into the growth media (ampicillin in one case and kanamycin in another). This does not comply with Section 4.32 of this present code. A relaxation is to be applied therefore for these specific cases. (Development work on other selective marker genes is underway).

c) Under the general requirements of this Code, media exposed to or containing antibiotics are not normally re-opened after incubation. Such a transformation protocol requires the sub-culturing of the modified bacteria from the plate into a broth so it may be grown up.

This then allows for the extraction and refining of the fluorescent protein. For these particular applications this requirement, not to re-open plates previously exposed to antibiotics, is also waived.

**Note on DNA and gene technologies:**

Practical work on such topics is expanding, albeit slowly, in Scottish schools and colleges. The pace of introducing this work has recently picked up. Such work is largely outwith the scope of this present code. For sound information and advice on safety in this area, teachers and students are best directed to relevant publications of the National Centre for Biotechnology Education (NCBE) and to Topic 16 "*Working with DNA*" in "*Topics in Safety*" (ASE - see Bibliography).

## APPENDIX 4

**Selected Micro-organisms and Culture Conditions for Large Scale Work at Levels 2 and 3, e.g. with bioreactors.**

Various inter-related factors influence the successful and safe operation of fermentation-type projects which involve the use of larger scale quantities of micro-organisms in bioreactors. Appropriate equipment and operational factors for this work have been described elsewhere in this document.

In Appendix 4, a range of selected micro-organisms is given, along with culture conditions which will favour the growth of a suitable large inoculum of the intended culture over the growth of possible undesirable contaminant organisms.

| Selected Organisms | Temperature Range $^{o}$C | Culture Conditions |
|---|---|---|
| *Acetobacter aceti* | 25-30 | Acidic medium containing ethanol |
| *Azotobacter vinelandii* | 30-35 | Nitrogen-free medium |
| *Chlorella (a green alga)* | 20-25 | Mineral medium exposed to light |
| *Gluconobacter oxydans* | 25-30 | Acidic medium containing ethanol |
| *Lactobacillus bulgaricus* | 41-45 | Acidic medium containing fermentable sugar(s) |
| *Lactobacillus lactis* | 41-45 | Acidic medium containing fermentable sugar(s) |
| *Methylophilus methylotrophus* | 30-35 | Mineral medium containing methanol |
| *Kluveromyces lactis* | 25-30 | Acidic medium containing fermentable sugar(s) |
| *Photobacterium phosphoreum* | 15-20 | High salt medium (optional, small amounts of glycerol) |
| *Saccharomyces cerevisiae* | 25-30 | Acidic medium or glucose solution |
| *Schizosaccharomyces pombe* | 25-30 | Acidic medium |
| *Vibrio natriegens* | 30-35 | High salt medium |

## Appendix 5 : Selected Bibliography


| | | |
|---|---|---|
| 1. | *Safety in Microbiology*<br>A Code of Practice for school and non-advanced further education.[2] | Strathclyde Regional Council (1989) |
| 2. | *Safety in Microbiology*<br>A supplement to the code of practice for schools and non-advanced further education: Post 16 student work at level 3.[3] | Strathclyde Regional Council (1991) |
| 3. | *Topics in Safety* (particularly Topic 15 "Microbiology and biotechnology") | ASE 3rd Edition (2001)<br>(0 86357 316 9) |
| 4. | *Safeguards in the School Laboratory* | ASE 10th Edition (1996)<br>(0 86357 2502*)* |
| 5. | *Be safe!* (Chapter 11 is useful for advice on level 1 work in Primary schools but it is not entirely consistent with this present code ). | ASE 3rd Edition 2001 |
| 6. | *Safety in Science Education* | HMSO (1996)<br>(0 11 270 915 X) [out of print] |
| 7. | *Preparing C.O.S.H.H. Risk Assessments for Project Work in Schools* | SSERC (1991) Revision due. |
| 8. | *Micro-organisms* for investigations in schools and colleges: revised listings. | SSERC Bulletin 194 (1998) |
| 9. | *Biology and Biotechnology : Microbiological Techniques*. (A set of laminated instruction cards for many basic microbiological techniques relevant to educational work). | Higher Still Development Unit (HSDU) and SSERC, 2001. |
| 10. | *Microbiological Techniques : An interactive manual for schools and colleges*. (A CD-ROM version of reference 9 based on web-browsing techniques). | SSERC in association with the Science and Plants for Schools (SAPS) Scotland Biotechnology Education Project, 2002. |


[2] Now superceded by this present document.
[3] As above.

**Appendix 6 : Useful addresses and other sources of advice**

Association for Science Education (ASE), College Lane, Hatfield, Herts. AL10 9AA
T: 01707 283000 F: 01707 266532 W: www.ase.org.uk
E: for list of contacts see - http://www.ase.org.uk/whois.html

ASE Scotland : www.asescotland.org.uk

CLEAPSS School Science Service at Brunel University, Uxbridge, UB8 3PH
*Tel:* +44 (0)1895 251496 *Fax/Answerphone:* +44 (0)1895 814372
*E-mail:* science@cleapss.org.uk (for UK member schools and colleges outwith Scotland).

Society for General Microbiology (SGM), Education Office, Marlborough House, Basingstoke Road, Spencer's Wood, Reading RG7 1AG E: education@sgm.ac.uk

Microbiology in Schools Advisory Committee (MISAC), see above for postal address. T: 0118 988 1835 F: 0118 988 5656 W:
www.microbiologyonline.org.uk/misac.html

National Centre for Biotechnology Education, School of Food Biosciences,
The University of Reading, Whiteknights, PO Box 226, READING RG6 6AP
T: 0118 9873 743 F: 0118 9750 140 E: NCBE@reading.ac.uk
W: www.ncbe.reading.ac.uk

SAPS Biotechnology Scotland Education Project, Institute of Cellular and Molecular Biology, The University of Edinburgh, Darwin Building, King's Buildings Campus, Mayfield Road, Edinburgh, EH9 3JR T:0131 650 7124 E:
kcrawfor@srv0.bio.ed.ac.uk
W: www-saps.plantsci.cam.ac.uk/index.htm

AND AT: Quest Biotechnology Laboratory, Dollar Academy, Dollar, FK14 7DU
T: 01259 743753 E: masmith@dollaracademy.org.uk

STS (Science, Technology and Safety) Support Services at SSERC, St Mary's Building, 23 Holyrood Road, Edinburgh EH8 8AE T: 0131 558 8180 F: 0131 558 8191
E: sts@sserc.org.uk W: www.sserc.org.uk (see, in particular, "Hotlinks" "Biology").

## Appendix 7 : Membership of the originating committees

This present code of practice is based on the earlier work of two working groups convened by the former Strathclyde Regional Council (see Bibliography). The memberships of those groups is given below.

**Originators of the 1989 edition** (asterisk denotes membership also of 1991 post-16 supplement group) :

Dr. O.A.M. de Almeida (then of Rosehall High School, Coatbridge)*

Dr J.G. Anderson (University of Strathclyde)*

Dr S.G. Deans (West of Scotland College of Agriculture)*

Mr J. Judge (then Adviser in Science Renfrew Division, Chaired 1989)

Dr M.F.Kohn (Microbiological Protection Adviser, University of Strathclyde)*

Mr A. Mackintosh (at that time, AHT Possilpark Secondary Glasgow)*

Mr B.J. Powlesland (Bell College of Technology, Hamilton)*

Mr J. Richardson (Director, SSERC)*

Dr J.W. Simms (Greenfaulds High School, Cumbernauld, Secretary 1989 and 1991 groups)*

Mr. J.M.Stafford (PT Biology Ravenspark Academy, Irvine and Chaired 1991 when Development Officer for Science, Ayr Division)*

**Post-16 group:**

Those marked * above plus:

Mr W.G. Fraser (then Education Officer, Strathclyde Regional Council)

Dr D. McGinlay (PT Biology, Merksworth High School, Paisley)

Mr D. McLaughlan (Project Officer, SSERC).