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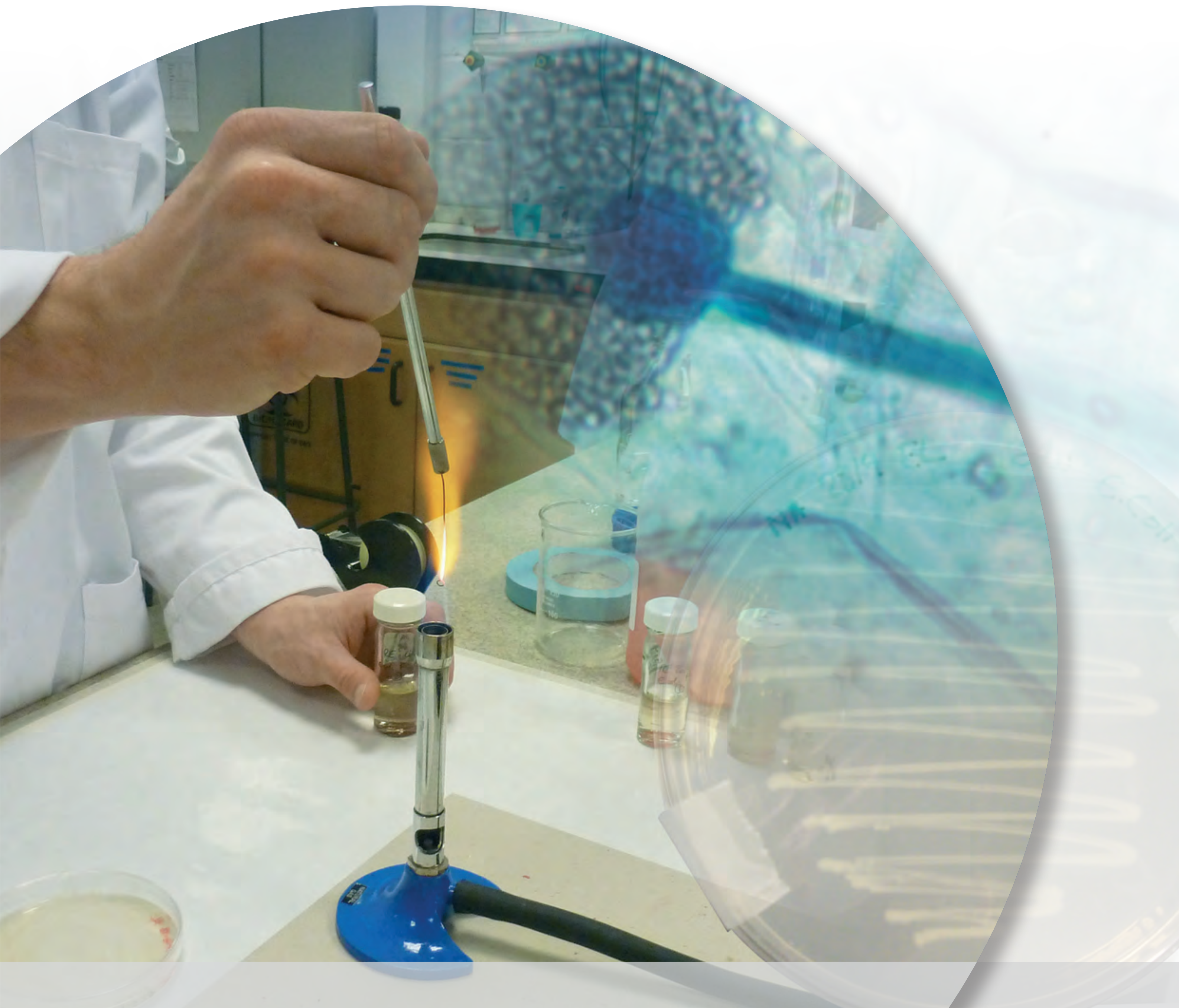

Safety in Microbiology

A Code of Practice for Scottish schools and colleges

Acknowledgement
This Code of Practice is based on 'Safety in Microbiology' and its post 16 supplement published by the former Strathclyde Regional Council. The contribution of the committee that produced that original guidance is gratefully acknowledged. It is a tribute to the quality of their work that much of that original Code of Practice has stood the test of time and is applicable to the present day. The contribution of Jim Stafford who chaired the original Strathclyde committee and who led the review that produced this current edition is gratefully acknowledged.

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Fourth edition published 2018
As a result of comment from practitioners, the layout of this 4th edition has been reorganised under headings for each level of work rather than under resources and good practice as in the previous editions. A subject index has been added, as well as information on training for technicians and teachers, maintaining microbiological cultures, the use of metal implements to transfer fungal mycelium and conditions under which students may undertake work at level 3. There have also been minor alterations throughout as a result of experience and feedback since the previous edition.

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Introduction

Practical work with micro-organisms

Practical work involving micro-organisms is of considerable educational value. It lends itself to investigative work in science and to the discipline of developing competence in practical skills. Microbiological skills are key components in the field of biotechnology and in the medical industries and services. The applications of microbiology are relevant to the everyday concerns of citizens in relation to food production, hygiene, health and waste management. It also allows for the evaluation and control of risk, a valuable life skill, as well as providing an insight into an area of science in which Scotland is a major global contributor in research and industrial production. In the 21st century responsible citizens will need to evaluate scientific issues related to microbiology and to develop informed views on the use and applications of micro-organisms.

Micro-organisms may be grown in Petri dishes, McCartney bottles and Universal containers. In addition some micro-organisms may be grown in bioreactors where there is a larger volume of liquid culture and growth conditions are suited to the growth of the micro-organism over any possible contaminants. A wide range of vessels from simple flask or bottle like containers to growth systems with elaborate sensing and control devices can be used as bioreactors.

The educational value of using bioreactors includes the following applications.

Applications which demonstrate:

- the use of growth vessels which simulate those used in commercial biotechnology;
- 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, data-logging and process control;
- batch, 'fed-batch' and continuous fermentation processes;
- the activity of enzymes or cells in an immobilised state or in an aqueous state.

Scope of the Code of Practice

This code is designed to cover most of the microbiology work likely to be carried out in Scottish Secondary schools. Some microbiology work carries very little, if any, risk and is therefore also suitable for primary schools (designated as level 1 work in this code). Equally much of the microbiology work covered by this code is appropriate for courses covered in further education.

Risk assessment

Practical work involving micro-organisms includes hazards (sources of harm or danger). These include the potential for:

- infection with a disease causing organism (pathogen), or
- 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. Individuals with severe allergic conditions may also be at particular risk if they encounter allergens to which they are sensitised.

It is the probability of harm being realised in practice that defines the degree of risk. Teachers and technicians must be aware of the risks in educational microbiological work and of the procedures that are to be followed in order to minimise them. This Code of Practice offers a set of preventive and protective measures produced as a result of model risk assessments. As such it can be adopted to meet the requirements of the COSHH Regulations and of other related health and safety legislation including Regulation 3 of Managing Health and Safety at Work Regulations 1999 - Risk Assessment.

It is the responsibility of the teacher or technician to identify the risks in any practical activity and then to consider adopting the Code of Practice as being suitable and sufficient to control these risks. By following the Code of Practice in this way a risk assessment is being carried out. If an activity falls outwith the guidance in the Code of Practice then an individual risk assessment for that activity must be carried out. It is not the intention of the code to be restrictive. In any event the employer's guidance on assessing risk and recording risk assessments should be followed.

Central principles of the Code of Practice

Central to the code 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:

- 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 technicians;
- age of the learners;
- available resources.

It is appropriate to define three levels of working with micro-organisms, which might be encountered in schools, according to the risks which they present and the type of training required before teachers and technicians should embark upon them *(note that these are not the same as the 'levels of containment' as used by professional microbiologists)*. In general, these levels are related to the educational stage of the learners. Thus in primary school only level 1 work should be attempted; in secondary schools, learners will normally be restricted to work at level 1 or level 2. In some SQA courses in the senior phase learners may, in addition, participate in level 3 work. Technicians and teachers who prepare materials for level 2 work should be trained to level 3 as they will have to carry out level 3 tasks.

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 technician may undertake will be limited by the training that the teacher or technician 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 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 learners other than as part of a supervised and structured learning activity.

1.12 If teachers or technicians 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. SSERC can provide advice on assessing such risks.

1.13 The use of bioreactors increases the potential risks involved in microbiology since larger volumes of liquid culture medium are used. For this reason, the choice of organisms (see appendix 4) and procedures which are permitted for use in bioreactors is more limited than that for small scale operations. Given these precautions, the educational value of these activities far outweighs the small degree of risk involved.

Work at level 1

2

Principles of 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 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 laboratory 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 be carried out only in rooms where food is normally prepared or where suitable clean and hygienic 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.

Resources required for level 1 work

2.8 Resources for good domestic hygiene are required.

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

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

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

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

2.13 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.

Good practice at level 1

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

2.15 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.

2.16 Containers used for microbiology work must be clean.

2.17 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.

2.18 The growth of micro-organisms should be carried out in covered containers (e.g. with aluminium foil covers or non-absorbent cotton wool plugs to prevent the escape of spores and/or to allow the release of any carbon dioxide produced).

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

2.20 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).

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

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

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

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

2.25 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.

Work at level 2

3

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.

Training required for work at level 2

3.1 For class laboratory work with learners at level 2 a science teacher does not require specialist (level 3) training. However, teachers may prefer and feel more confident in managing level 2 laboratory class work if they are trained to level 3.

3.2 Support for science teachers with limited experience of microbiology could be provided in a short in-school training session from a more experienced technician or teacher colleague. Such support could based on the SSERC *Microbiological Techniques* [1] resource materials.

3.3 Teachers of level 2 classes must be trained in dealing with spillages.

3.4 In order for level 2 class laboratory work to be safely carried out, personnel trained at level 3 must be available to carry out the preparation, maintenance and disposal level 3 tasks necessary to support level 2 work.

Principles of work at level 2

3.5 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 Appendices 1 and 2 can be used.

3.6 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.

3.7 Normal laboratory facilities (including hand washing) and specialised facilities for the preparation of media and disposal of cultures are required.

3.8 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.

3.9 Protective clothing is not essential.

3.10 All sterilisation required for level 2 work is a level 3 task.

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

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

3.13 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.

3.14 At level 2, samples may be taken from a freshly prepared yeast suspension with a clean cotton bud to inoculate sterile solid media suited preferentially to the growth of yeast. Learners may also inoculate sugar solutions with yeast cells for large scale liquid culture work.

3.15 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.

[1] https://www.sserc.org.uk/health-safety/biology-health-safety/microbiological-techniques/.

3.16 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 [2] 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 [3] (e.g. from a pond or field).

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

Resources required for level 2 work

3.18 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.

Hand washing facilities, including sink, soap, water, paper towels and a bin must be available. Suitable protective clothing (e.g. lab coat) is not required for level 2 work.

3.19 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.

3.20 Any discard jars, used for contaminated used equipment, must contain freshly diluted *Virkon* at a concentration of 1% w/v.

3.21 Disinfectants which contain available chlorine [e.g. *Chloros*, sodium chlorate (I) [hypochlorite] or bleach] may be used in suitable dilution to surface sterilise plant material (e.g. beans, peas).

3.22 *Virkon* must be available for treating spillages and for adding to bioreactors after use.

3.23 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.

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

3.25 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. Sterile disposable plastic loops may also be used.)

3.26 Proprietary cotton buds may be used as inoculation swabs to sample the environment and to sub culture freshly prepared yeast suspension.

[2] The classic ‘finger dabs’ experiments on washed and unwashed human hands may be carried out provided that only sterile plates are used and they remain closed after inoculation and through to disposal.
[3] The anaerobic conditions in mud may suit the growth of pathogenic organisms.

3.27 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.

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

3.29 Reactor vessels must be constructed from robust material.

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

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

3.32 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 carboy.

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

Good practice at level 2

General

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

3.35 Techniques for handling microbiological materials are described in *Microbiological Techniques* published by SSERC [4].

3.36 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.

3.37 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.

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

3.39 Exposed cuts should be covered with waterproof dressings.

3.40 Long hair should be tied back.

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

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

Cultures

3.43 Where micro-organisms from Appendices 1, 2, 3 or 4 are used, they must be obtained from an approved supplier. An approved supplier is one who can trace the provenance of the supplied culture back to a national culture collection that is true to type.

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

3.45 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.

3.46 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.

3.47 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.

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

[4] https://www.sserc.org.uk/health-safety/biology-health-safety/microbiological-techniques/.

3.49 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.

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

3.51 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*.

3.52 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*, *Penicillium* and *Botrytis* which produce large numbers of easily dispersed spores and to which allergic reactions may occur.

Media

3.53 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.

3.54 Antibiotics should not normally be incorporated into growth media and only commercially produced paper impregnated antibiotic discs should be used [5]. (But see Appendix 3 on the use of antibiotics in specific protocols).

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

3.56 Media for use in Petri dishes should be cooled in a thermostatically controlled water bath at 55°C before pouring. The necks of media containers should be flamed before pouring.

3.57 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.

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

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

Inoculation

3.60 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.

3.61 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. Plastic disposable loops can only be used for one transfer and then must be placed in a discard jar containing *Virkon*.

3.62 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.

[5] The selected micro-organisms do not include species that produce antibiotics. To carry out practical work with such species would require a separate risk assessment. SSERC would be happy to assist with such an assessment.

3.63 Cotton buds must only be used for sampling from the environment and must not be used for culture transfer work other than for a freshly prepared yeast suspension. 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.

3.64 When using metal implements such as scalpels, mounted needles, cork borers or forceps to cut and transfer fungal mycelium to Petri dishes or slopes the following procedure should be observed:
   a) Dip the implement in alcohol.
   b) Pass the implement through a Bunsen flame and allow the alcohol to burn off.
   c) Carry out the transfer operation.
   d) Repeat the alcohol flame procedure with the implement.
   e) Should fragments of agar visibly adhere to the implement, place it in a disinfectant discard jar prior to autoclaving rather than using the alcohol flame procedure.
   f) Exercise caution to keep the alcohol container a safe distance from any Bunsen flame. Should there be concern over the likelihood of the alcohol igniting, it would be safer to sterilise the implements by autoclaving. After use place the implements in a disinfectant discard jar prior to autoclaving.

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

3.66 Once inoculated, the lids of McCartney bottles or Universal containers should be tightened.

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

3.68 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

3.69 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.

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

3.71 Incubations are not normally to be carried out above 30°C; exceptions to this are given in Appendices 3 and 4.

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

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

Bioreactors (fermenters)

3.74 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%) of the total volume of the medium.

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

3.76 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.

3.77 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.

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

3.79 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.

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

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

3.82 Unused bioreactor ports must be effectively stoppered.

3.83 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.

3.84 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).

3.85 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.

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

3.87 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

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

Disposal

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

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

Spillages

3.91 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 30 minutes. A person trained for level 3 work must be informed immediately.

3.92 Small scale spillages (e.g. from McCartney bottles or Universal containers, or breakages of Petri dishes) must be covered with paper towels soaked with *Virkon* 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.

Work at level 3

4

Work at level 3 can involve additional micro-organisms and a wider range of inoculation and transfer techniques.

Training required for work at level 3

4.1 For level 3 work technicians and teachers are required to be trained to level 3. They must have undertaken and achieved the competence standards of the SQA accredited SSERC course Safety in Microbiology for Schools (level 3). Senior phase students may be trained to carry out a range of level 3 tasks for specific experiments or project work. Such students must be supervised by a teacher or technician trained to level 3.

Principles of work at level 3

4.2 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) preparation of cultures for class use;
d) maintenance of stock sub-cultures;
e) sampling from bioreactors;
f) sterilisation and disposal of cultures;
g) sterilisation of used equipment;
h) management of incidents of spillage;
i) staining of incubated plates (e.g. starch agar [6]).

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

4.4 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.

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

4.6 Samples taken from solid cultures of micro-organisms from Appendices 1, 2, 3 and 4 can be used to inoculate sterile solid or sterile liquid medium.

4.7 Samples taken from liquid cultures of micro-organisms from Appendices 1, 2, 3 and 4 can be used to inoculate sterile solid or sterile liquid medium'

4.8 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.

Resources required for level 3 work

In addition to the resources required for level 2 work, personnel trained at level 3 must have access to the following resources, in order to prepare for, and support, level 2 work.

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

4.10 Suitable protective clothing (e.g. lab coat).

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

[6] Uninoculated starch agar plates used for enzyme studies may be opened and stained by students. Where starch agar plates have been inoculated with a micro-organism, only a level 3 trained person may open and stain the plates.

4.12 A spillage kit containing:
- a screw-capped bottle containing a measured quantity of *Virkon* 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.

Good practice at level 3

The good practice in using microorganisms at level 2 applies equally to work at level 3.

Sterilisation

4.13 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.

4.14 Items of equipment which cannot be steam sterilised can be decontaminated by the use of chemical disinfectants, e.g. a hypochlorite solution or *Virkon*.

4.15 Glassware which cannot be conveniently autoclaved may be sterilised by dry heating in an oven maintained at 160°C for 2 hours.

4.16 Sterile media may be prepared by a senior phase student for specific experiments or project work under the supervision of a teacher or technician trained for work at level 3, but not on a routine basis.

Sub culturing

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

4.18 To maintain microbiological cultures, the purchased culture should be sub cultured onto two agar slopes. One of these cultures should be used to prepare cultures for class use and the other retained as a source for future use or for use if contamination is observed. When using this second culture, again prepare two agar slopes as before; one for preparing cultures for class use and one to be retained for further use [7].

4.19 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).

4.20 A senior phase student may open a culture on solid media that they have inoculated and incubated themselves only after the following conditions have been met:

a) The student's culture 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.

[7] Maintaining microbiological cultures in school https://www.sserc.org.uk/wp-content/uploads/Publications/Bulletins/248/SSERC_bulletin_248p8.pdf.

Inoculation

4.21 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 *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 a sterile disposable type).

4.22 Transfer of fungal mycelium to Petri dishes or slopes using metal implements should be carried out following the procedures outlined at 3.64.

Disposal

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

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

4.25 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.

4.26 Bioreactor contents inoculated with appendix 4 microorganisms should be treated with a suitable quantity of *Virkon* to provide a working solution of 1% in the bioreactor. The contents should be held for at least one hour prior to flushing away with a large volume of water.

4.27 Senior phase students may autoclave and dispose of cultures under the supervision of a teacher or technician trained for work at level 3, but not on a routine basis.

Spillages

4.28 Spillages should be treated as below.

a) Action as detailed in 3.91 and 3.92.
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.
e) The tongs or dustpan must be autoclaved or covered with a hypochlorite solution, or a solution of *Virkon*, for 24 hours.

4.29 Gross spillages must not be dealt with by learners. Learners 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.

Appendices

5

Selected micro-organisms for Scottish schools and colleges

The micro-organisms listed in Appendices 1 to 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 published by the Microbiology Society, the National Centre for Biotechnology Education and the Association for Science Education (*Topics in Safety*, Topic 15: Microbiology). The fuller listings also give points of educational use or interest and comment on the ease with which organisms can be cultured and maintained. These fuller lists can be accessed on the Microbiology in Schools Advisory Committee (MiSAC) web site [8].

All micro-organisms named in Appendices 1-4 must be obtained from an approved supplier or obtained from a recognised UK culture collection (usually, but not always, more expensive). An approved supplier is one who can trace the provenance of the supplied culture back to a national culture collection that it is true to type.

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. Schools and colleges wishing to use micro-organisms not included in these lists, or to depart from the procedures in this code, must first carry out an appropriate risk assessment. SSERC is happy to assist in that process and to consider additions to these lists.

APPENDIX 1

Selected organisms for work at level 1

- Bread making or brewer's yeast (*Saccharomyces cerevisiae*)
- Dried yoghurt cultures (bacteria used to make yoghurt)
- Blue-green algae
- Green algae
- Free living protozoa
- Lichens
- Slime moulds

[8] http://www.misac.org.uk/PDFs/MiSAC_suitable_and_unsuitable_micro-organisms.pdf.

APPENDIX 2

Selected organisms for work at levels 2 and 3

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

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

Bacteria
Acetobacter aceti
Agrobacterium tumefaciens
Azotobacter species
Alcaligenes eutrophus
Bacillus megaterium
Bacillus stearothermophilus
Bacillus subtilis
Cellulomonas species
Chromatium species
Janthinobacterium lividum (also called *Chromobacterium lividum*)
Escherichia coli (strain B or strain K12)
Gluconobacter oxydans
Lactobacillus species
Micrococcus luteus (also called *Sarcina lutea*)
Micrococcus roseus
Methylophilus methylotrophus
Pectobacterium carotovorum (also called *Erwinia carotovora*)
Photobacterium phosphoreum
Pseudomonas fluorescens
Rhizobium species
Rhodopseudomonas palustris
Spirillum serpens
Staphylococcus 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

APPENDIX 3

Selected organisms for specific protocols

- *E. coli* strain B
- *E. coli* strain K12
- Bacteriophage

Notes on specific protocols using *E. coli*

1) Some *E. coli* strains are associated with health hazards. *E. coli* strains B and K12 are widely used laboratory strains that have lost the ability to colonise the human gut and so present a reduced risk compared to other strains of *E. coli*. These two strains of *E. coli* should be suitable for all projects which require the use of *E. coli*.

2) *E. coli* strain B is a suitable host for T 'phages, T1-T7, and λ 'phage.

3) *E. coli* strain K12 is suitable for self cloning experiments and for transformation protocols using plasmids with the GFP (green fluorescent protein) gene, for example pGLO. These protocols may involve incubation temperatures of 37°C and the use of media containing antibiotics. Although these conditions are outwith the Code of Practice, a suitable risk assessment can make such practical work suitable for schools. Suitable control measures to control risk are usually included in such protocols. SSERC can provide advice on suitable protocols and risk assessment.

APPENDIX 4

Selected 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 (see below) 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.

Appendix 4

Selected organisms	Temperature range °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

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- Suitable and unsuitable microorganisms, Microbiology in Schools Advisory Committee (MiSAC), http://www.misac.org.uk/PDFs/MiSAC_suitable_and_unsuitable_micro-organisms.pdf.

- Topics in Safety, Association for Science Education, Topic 15 Microbiology.

- Topics in Safety, Association for Science Education. Topic 16 Working with DNA.

- Safeguards in the School Laboratory, Association for Science Education, 11th edition, 2006 ISBN 978-0-86357-408-5. Chapter 13 Biological Hazards.

- Be safe! Association for Science Education, 4th edition, 2011, ISBN 978-0-86357-426-9.

APPENDIX 6

Useful organisations and their websites

- **Microbiology in Schools Advisory Committee (MiSAC)** http://www.misac.org.uk/

- **Microbiology Online**
  http://microbiologyonline.org/

- **Microbiology Society**
  http://www.microbiologysociety.org/

- **National Centre for Biotechnology Education, University of Reading**
  http://www.ncbe.reading.ac.uk/

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