SCOTTISH SCHOOLS SCIENCE

EQUIPMENT RESEARCH

CENTRE

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Introduction

If the strength of feeling for 'disintegrated science' to which we referred in our last bulletin is anything to go by, then this issue, dealing as it does exclusively with microbiology will be of only throw-away value to quite a number of chemists and physicists. If this be so, then we make a plea that it be thrown not in the waste paper basket, but onto the desk of a colleague in such subjects as home economics, anatomy, physiology and health, or agriculture. They may find it of interest, and possibly of value. However, we would ask the non-biologists not to ignore this issue. In many schools they may find themselves involved with simple practical microbiology, and need to be familiar with current recommended practice.

We intend to provide for home economics advisers in the regions, or to health and safety officers where there are no home economics advisers, a shortened version of the microbiological article below. This would give guidance on what level of microbiological work should be undertaken in home economics departments, and it would be left to regional advisers to decide whether and how this could be transmitted to the schools.

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Our logo competition had a small entry from which Mr Jack Firth, art adviser in Lothian Region decided that the entry by Mr A Steven, Drummond High School, Edinburgh was the winner. The logo will appear in the not too distant future, although as other matters connected with the bulletin format have still to be decided, we cannot say which number will see the change. If any of the unsuccessful entrants wish to have their entries returned would they please 'phone us, as otherwise they will not be returned.

Biology Notes

Summary of the main recommendations

(i) Thoughtful microbiological practical work in schools can have great educational value. Given a sensible safety policy, this educational value can easily outweigh the level of risk then involved. It seems reasonable to recommend that such practical microbiology should continue in schools and indeed, be encouraged.

(ii) In general, work with bacteria and fungi for younger, secondary pupils should be restricted to simple agar-plate work, the plates remaining closed after inoculation. Sub-culturing and other 'transfer' work, requiring sound aseptic technique should, usually, be restricted to pupils over 16, supervised by an experienced biology teacher.

(iii) Cultures should be obtained from a reputable source (e.g. a major biological supply house or a Culture Collection) and would normally be of organisms named in the list issued by the Microbiological Consultative Committee.

<u>Why study microbiology</u>?

The nature of the growth, reproductive capacity and biochemistry of many micro-organisms makes them of great economic, social and medical importance. The fundamental rules of personal, public, and domestic hygiene rest on an understanding of the characteristics of these organisms. Micro-organisms possess many obliging features which make them ideal subjects for practical exercises in schools. Unlike many organisms they do not necessarily have to be maintained over long periods, don't have to be fed and watered at weekends and can be killed without fears of emotional outcries!

The ASE-convened Working Group is convinced of the general educational value of thoughtful, microbiological practical work in schools and of the need for every pupil to possess a basic knowledge of the biology of these organisms.

<u>What are the risks</u>?

Some of our pupils have to be convinced of the necessity for simple rules of hygiene, food preparation etc. Micro-organisms have to be seen to be believed. Observation using a microscope can be difficult. Second hand evidence of pictures and photographs may be unconvincing, and can impart little of the dynamics of growth and reproduction. Therefore the work will involve the culturing of organisms so that numbers are increased and colonies and growths can be observed. Most of the risks arise from this necessity for an increase in the numbers of organisms.

Microbiological work in schools is not intentionally concerned with known pathogens. In addition, the human body has a battery of weapons to help ward off infection. However, safety measures are absolutely essential for several reasons:

(a) The dividing line between pathogens and non-pathogens cannot be sharply drawn. The blurred area between them may never be clear and has extended with time. A number of school texts have recommended the routine use of 'harmless' organisms which we now recognise as potential human pathogens.(1)

(b) The possibility of chance contamination by pathogens exists. Culturing procedures designed to concentrate relatively harmless organisms for study can also concentrate pathogens that normally occur in the environment at sub-infective levels.(2)

(c) The properties of micro-organisms are not fixed. Non-pathogenic strains can and do mutate, becoming potentially harmful. Bacteria can develop resistance when exposed to antibiotics. Resistant strains may then cause illness either directly or by transferring their resistance to other, pathogenic bacteria.

Medical journals contain scattered reports of relatively harmless saprophytes becoming pathogenic. It is reported that such occurrences seem commoner since the introduction of the sulphonamide drugs and antibiotics. The bacteria involved include some hitherto used in schools: <u>Serratia marcescens</u>; <u>Pseudomonas aeruginosa</u>; and <u>Chromobacterium violaceum</u>. The organisms involved usually have marked resistance to the commoner antibiotics. In any potentially hazardous situation, the dangers must first be recognised before they can be controlled or eliminated. This requires knowledge and training. The greatest hazards arise from ignorance of the inherent dangers in any work designed to concentrate micro-organisms. Given a suitable

choice of organisms, sensible precautions and a level of work appropriate to the experience and training of pupils and teacher, the educational value of practical microbiology can far outweigh the relatively small risks then involved.

'Levels' of work

In school microbiology there are three levels of work which can be identified:

(1) Work with organisms carrying little, if any, risk where faulty technique may lead to the demise of the cultures but is unlikely to harm pupils or teachers, e.g. with algae, non-pathogenic yeasts and protozoa.

(2) Work where certain prepared media are exposed to the environment or are inoculated with material from the environment or from a purchased culture. However, isolates are not made and cultures are not transferred. Consequently advanced aseptic techniques are not needed. Work at this level is frequently met with in 'combined' or 'integrated' science courses. Given general supervision by an experienced biology teacher and simple, school-based in-service training there is no reason why secondary science teachers without formal qualification in biology may not safely carry out this work.

(3) Work with cultures of fungi and bacteria where sub-culturing and transfer procedures are required as matters of routine. Good aseptic technique is needed. This can only be acquired after thorough training and practice. This work would not usually be done or supervised by non-specialist staff unless they had attended a suitable course of in-service training. Normally the students involved would be sixth formers.

Little needs to be said about the work at level (1). Culture media are used but are generally of a type not likely to encourage growth of pathogens. All micro-organisms should be treated with caution and simple rules of hygiene be observed. Some authors recommend the use of faecal material as an 'enrichment' in the preparation of infusoria etc. Under no circumstances should this recommendation be heeded, because of the possibility of culturing pathogens from the faecal material.

Work at level (2) carries some risks but these should be minimal if certain simple precautions are taken. In schools work at this level usually involves exposing prepared plates of nutrient agar to various sources of micro-organisms. If class discipline cannot be relied on, consideration should be given to restricting the work to teacher demonstration. This is a regrettable but, with some pupils, necessary consideration.

The essential difference between this work and level (3) lies in the fact that microbial growths are not subcultured after incubation. The cultures, after incubation, are merely observed and recorded after which they are destroyed. Safety precautions for work at this level are given below.

At level (3) inoculation, sub-culturing, dilution plating and other procedures may be carried out routinely. These procedures involve a number of operations where microbial aerosols may form. These aerosols consist of fine droplets and particles contaminated with cells or spores of organisms. They can be formed whenever the

surface of a liquid is broken or when dry material is crushed, ground or shaken. Once formed they may cause widespread, persistent contamination. The particles are small enough to penetrate the respiratory passages where what would normally be a sub-infective dose may cause illness.(3)

Bacterial and fungal spores are ubiquitous. Unless technique is good, experiments can be ruined. Far worse, chance contamination by and the isolation of pathogens may occur. The importance of theoretical and practical training should not be underestimated. Anyone, sixth year pupil or teacher, carrying out work at this level should adhere strictly to the correct methods of handling cultures and apparatus until these become habitual. Good aseptic technique is paramount. A number of additional precautions are therefore needed for work at this level.

Preparation of equipment and media - all levels of work

(i) Except for the simplest fermentation experiments with yeasts, chemical disinfection should be reserved for the treatment of spillages and contaminated used equipment. It should not be used for the preparation of equipment.

(ii) U.V. radiation is not appropriate for general sterilisation of glassware or other equipment. It will not penetrate glass or films of dirt etc. U.V. lamps will continue to emit visible radiation after emissions at the germicidal wavelength have ceased.(4)

(iii) Glassware such as pipettes is best sterilised by dry heat in an oven at 160°C for at least one hour. One hour is the holding time. It is essential to allow an additional warm-up period for oven and contents to reach 160°C. This heating up period may itself be as long as 1 hour.(3) The pipettes are wrapped in paper or aluminium foil and placed in a metal pipette canister. Square canisters are better than round ones which tend to roll.(5)

(iv) Liquids and articles which would be damaged by dry heat at 160°C are sterilised by steam at 121°C (103kNm^{-2} or 15lbin^{-2} steam pressure, i.e. one atmosphere above normal atmospheric pressure). The holding time under these conditions should be at least 15 minutes. In schools autoclaves are invariably of the non-jacketed, 'pressure-cooker' type. Indeed the 'autoclave' may actually be a domestic pressure-cooker. These vertical, portable laboratory autoclaves are adequate for all normal school work but their limitations should be recognised. Their main disadvantage is that there may be inefficient removal of air, and their small size is a temptation to overloading.(3)

Air has an important influence on the efficiency of steam sterilisation. For example, if all the air is removed from the vessel, saturated steam at 103kNm^{-2} (15lbin^{-2}) has a temperature of 121°C. With only half the air removed, the temperature of the air-steam mixture is only 112°C.(3)

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

(a) a heating up period to allow the water to come to the boil;

(b) a period of vigorous free steaming to expel air from containers;

(c) an addition to the holding period, possibly <u>but exceptionally</u> as much as an extra 20 minutes, if the load includes certain 'difficult' materials;

(d) a cooling period, rapid cooling under a tap or by other means being inadvisable. The cooling period increases the time of exposure to steam and may be necessary for the effective sterilisation of certain materials.(6) In any case, rapid cooling may lead to glassware cracking or liquids boiling over and being wasted. It can be very dangerous to open an autoclave before the pressure has dropped to atmospheric (when the temperature inside will be ca. 80°C). The sudden change in temperature caused by the opening before the pressure has been allowed to fall, has been known to cause violent cracking of glass containers.(3) Serious scalds and burns have occurred because this hazard was not appreciated.(7)

'Difficult' materials referred to under (c) would not be frequently met with in normal school work. We have in mind materials like dry soil which will contain heat resistant spores and will allow steam to penetrate only very slowly. Contaminated cloth can also be difficult because the displacement of air can be a problem. However even very exacting samples such as soil caked on tightly rolled and packed lint have been shown to be reliably sterile after a 35 minute holding time in a domestic pressure cooker.(6) <u>For standard media and recommended non-pathogenic organisms, a 15-20 minute holding period will be effective</u>. Should there be any doubt, the holding time should be increased. The other simple precautions necessary for the proper use of school laboratory autoclaves are detailed in the Appendix.

(v) When agar media are being prepared the agar is added to cold water, allowed to soak and then brought to the boil. Agar should never be added to boiling water, the violent frothing which results has caused serious scaldings.

(vi) After use a pressure cooker or autoclave should be cleaned out. Nutrient materials spilled or leaked from containers into the autoclave will otherwise form an excellent medium for microbial growth. Although sterile at the time of the spillage it will not remain so for long.

<u>Techniques and precautions for work at Level (2)</u>

The educational value of these activities far outweighs the small degree of risk involved when the following safety precautions are observed:

(i) Before starting work, wash the hands and dress any cuts with waterproof dressings. Before leaving the laboratory hands should be washed with soap and hot water.

(ii) Do <u>not</u>, under any circumstances, culture microbes from potential sources of pathogens such as human (or animal) blood, mucus, pus from cuts, urine or faecal material (including surfaces in toilets etc.).

(iii) All hand to mouth operations should be banned. There should be no eating, drinking or smoking in the laboratory. Wax pencils or self-adhesive labels should be used for labelling and not the gummed type of label.(3)

(iv) Technicians or teachers handling media etc. should wear suitable laboratory coats and sensible arrangments for

storage and laundering made. Careful consideration should be given to the need for pupils to wear laboratory coats.

(v) No experiment should be performed which involves the deliberate contamination of the skin or other parts of the body.(8) (Unless 'level 1' type organisms such as non-pathogenic yeasts are involved).

(vi) Cultures should be incubated at an appropriate temperature. Incubation at 37°C, a temperature recommended by many authors for general bacteriological work, tends to select organisms adapted to man's body temperature. Growths will occur satisfactorily, albeit more slowly, at somewhat lower, room, temperatures. Incubation at high temperatures can isolate thermophilic fungi some of which produce infective spores. All of the recommended organisms listed at the end of this article will grow satisfactorily at 25°C or below.

(vii) In this elementary work, anaerobic culture should be avoided since there is a danger of isolating anaerobic, enteric and other pathogens. The use of certain enrichment media such as blood agar, bile salts etc. is also inadvisable at this level because it increases the chances of isolating pathogenic organisms.

(viii) In general broth cultures are more difficult to handle safely. If used at all at this level the work should be restricted to demonstration by an experienced teacher.

(ix) Cultures should not be centrifuged. Centrifuging is a common cause of aerosol formation. School centrifuges lack capped tubes and other devices to contain any aerosol which may form.

(x) Petri-dish cultures should be incubated base uppermost so that any condensation will drip into the lid. The cultures should be properly labelled (see iii).

(xi) Natural sources such as soil, water and raw milk can all contain pathogens. As a general rule ALL PLATES SHOULD BE SEALED AFTER INCUBATION. If plates are fully sealed by taping the circumference growth may be atypical because of reduced oxygen content. Apparently some tapes are bactericidal and may prevent growth during incubation.(5) It is probably better during incubation to use several smaller pieces of tape, at the sides of dishes. After incubation dishes should be sealed by taping round their circumference.

(xii) Condensation in Petri-dishes is avoidable. Excessive condensation is a sign that the medium was too hot when poured. If a great deal of condensation does occur during incubation, making it difficult to see the culture, a dry, sterile replacement lid should be fitted by the teacher or technician and the dish resealed. The operation should be rapid but careful and carried out over a piece of lint moistened with a suitable disinfectant since aerosols may be formed (recently introduced disinfectant-impregnated 'Wipex' cloths may prove more convenient than moistened lint - see also section on spillages). The old lint should be disposed of by the methods described below.

(xiii) Inspection of open culture dishes by pupils is rarely, if ever, necessary at this level. If it is allowed, the organisms should be killed by placing a filter paper moistened with a few drops of 40% methanal (formalin)

solution (care) in the dish 24 hours prior to examination.[9] It is our opinion that culture plates which have been used in any experiment involving antibiotics should be kept sealed at all times.

(xiv) Microbiological materials should never be kept in refrigerators used for home economics etc. Similarly it is part of good laboratory practice that food for human consumption should never be kept in laboratory 'fridges. It also follows that for microbiology practicals, carried out as part of a home economics course the laboratory facilities of the science department should be used. For the preparation of culinary products for human consumption, involving the use of bacteria or fungi, the proper facilities are those of a good kitchen. These facilities are to be found in the home economics department.

(xv) Pupils should realise that all contaminated equipment must first be sterilised even if it can be washed and re-used. Special areas and containers should be set aside for contaminated apparatus and materials. Spillages of contaminated material on bench, floor or person must be reported to the teacher and swabbed with a suitable disinfectant (see page 9).

<u>Additional precautions for work at level (3)</u>.

Work at this level may involve sub-culturing and transfer work and more sophisticated aseptic techniques are required. In addition to the safety precautions outlined for level (2) the following points should be noted:

(i) The work area should ideally be on a hard swabbable bench surface such as plastic laminate and away from doors, windows and other direct sources of draughts. Before starting work swab the bench with a suitable disinfectant (i.e. a clear phenolic, 'hypochlorite' or an ampholytic surfactant - see below for details).

(ii) Working close to a bunsen burner, where the updraught will prevent organisms falling onto apparatus, gives protection to both work and worker.

(iii) A good deal of technique is associated with the use of the inoculating loop. Loops can be easily made by bending 24 s.w.g. nichrome wire round a match stick, making sure that the loop so formed is fully closed. The overall length of the wire including the loop should be no more than 50mm. This is to minimise vibration and flickering of material from a charged loop. Loops should be attached to a metal 'chuck' type holder and not to glass rods. This is because flame sterilisation should include the lower part of the handle. When the handle is a glass rod it is likely to shatter.

(iv) Any instrument introduced into a culture must first be sterilised. Loop and lower handle are heated to red heat in a roaring bunsen flame. The wire is allowed to cool, or can be quenched in sterile water, before use. Alternatively the wire can be dipped in ethanol and passed through the flame to burn off any excess. Direct flaming of a wet loop can cause spluttering. Material which spits from a charged loop may not have been sterilised. This can be avoided by immersing the contaminated loop in a beaker of boiling water before flaming. Kampff pattern burners are

available which contain and sterilise any material which spits from loops, but these are more expensive than the ordinary bunsen.

(v) The mouths of culture tubes, McCartney bottles etc. should be flamed when removing caps or plugs, and the flaming should be repeated before replacement. Plugs and caps should not be placed on the bench. With practice it is possible to manipulate tubes, plugs and loop without any of them leaving the hands. If tubes or caps cannot be handled conveniently they can be placed on a ceramic tile which has been swabbed with a suitable disinfectant. Always support culture tubes and similar glassware in a rack, preferably a plastic coated wire one, never prop them up or lie them down on the bench.

(vi) Lids of Petri dishes should be opened just enough to allow the inoculating tool to enter and be manipulated. Lids should be opened for the minimal amount of time necessary for the particular operation to be performed. The lid should be held open at an angle, the opening facing away from the worker. Occasionally, for certain operations, a lid may have to be removed completely. It should be placed, inner surface down, on a clean surface such as a disinfected ceramic tile, or on a piece of lint moistened with disinfectant. The lint should only be moist, when fallen drops will diffuse into it. If it is saturated then an aerosol may still be produced.(9)

(vii) Where 'transfer chambers' are used their limitations should be realised. The chambers on the schools market can help to cut down the general level of contamination of a laboratory by 'weed' micro-organisms. They can also provide a clearly delineated 'clean' area for sixth year work in laboratories that have to be used by other forms. However they are <u>not</u> designed to give protection against potential pathogens. The use of such a chamber does not give protection against the consequences of poor technique. In the event of a spillage, any aerosol or spore cloud formed may be concentrated right under the nose of the operator.

(viii) Pipettes, including Pasteur pipettes, have their wide end plugged with cotton wool in an attempt to keep them dust and microbe free. Plugs can be easily penetrated by organisms in liquid suspension. A wet plug will obstruct air flow and can be sucked out followed by a gush of liquid. For this reason, pipettes are never used in the mouth. A variety of simple devices is available for drawing fluid into a pipette. For quantitative work either sterile disposable syringes or graduated glass pipettes, heat-sterilised in aluminium foil and operated by an autoclavable rubber bulb or similar device should be used.

(ix) Where 'stock' cultures are kept they should be checked for contamination before use. Plate out a sub-culture and examine for mixed growth. If mixed cultures are found, destroy the stock by autoclaving it and buy in fresh stock.(8)

<u>Treatment of contaminated equipment - all levels</u>.

(i) The safest disposal procedure is to use autoclavable disposal bags. Plastic materials such as disposable Petri dishes should be placed in the bag - this is then closed lightly with a wire cored tie and autoclaved.

Contrary to the advice of many authors, the bag should not be actually sealed until after autoclaving. The sterilised bag and contents should then, ideally, be incinerated. It may, as a second best, be passed into the normal refuse disposal system. Retainable glassware can be autoclaved directly and then washed and stored. Before re-use such glassware should be sterilised by autoclaving.

(ii) If the only autoclave available is a domestic pressure cooker, then disposal of large amounts of material can mean having to autoclave in several batches. This can be tedious. It is sometimes suggested that in these circumstances culture plates etc. can be placed in a bucket of disinfectant and left to soak, at least 18 hours, before disposal.[8] It must be stressed that this is a less safe procedure than autoclaving and is best not adopted as the routine method. This is because it can be difficult to remove entrapped air. Unless all the trapped air is removed disinfectants may be ineffective.[7] Chemical disinfection before disposal should use the disinfectants specified below for pipettes.

(iii) Contaminated pipettes should be placed in a container with a suitable disinfectant, i.e. those relying on available chlorine, the 'clear' phenolics, or an ampholytic surfactant for their action.

Solutions of the first type should contain at least 1,000ppm available chlorine. In dirty conditions up to 10,000ppm may be required. They may be made up using sodium chlorate(I) (hypochlorite), 'Chloros', 'Domestos' or 'Milton'. Because the concentration of chlorate(I) varies in proprietary preparations, the manufacturer's instructions should be followed. 'Chlorine' solutions are corrosive and irritant. Protective gloves should be worn and solutions kept off any metallic parts of apparatus. Solutions give off chlorine readily and lose effectiveness and so should be made up freshly each time they are used. In fact, all disinfectants are of doubtful value unless they are freshly prepared and dilutions accurately made.[3] It follows that diluted disinfectants should not be stored.

The clear phenolics (e.g. 'Clearsol', 'Hycolin', 'Printol', 'Stericol') are not much inactivated by organic matter and do not affect metals. In their correct use dilutions they are unlikely to damage skin, although prolonged contact should be avoided. They have a wide antibacterial spectrum, but are only weakly active against spores.[10] Use all these phenolics at the manufacturer's suggested dilutions. Lysol and other cresolic disinfectants are very toxic, caustic and non sporicidal and should not be used in school laboratories.

'Tego MHG', Gerrard 'ASAB' and Harris 'BAS' are ampholytic surfactants and can be used in 1% solution (freshly made).

The pipettes are soaked in the disinfectant for at least 18 hours and, ideally, are then autoclaved together with the pipette pot in which they have soaked. They are then washed, placed in pipette cans and sterilised by dry heat before being used again. Some graduated pipettes are too large to fit in the domestic pressure cookers used in many schools. These larger pipettes will have to be washed immediately after soaking overnight in disinfectant and sterilised directly by dry heat.

Treatments of spillages - all levels

Spills of cultures on bench or floor may result in the formation of a contaminated aerosol cloud. Bending over the spillage to mop it up immediately will bring the face into this cloud. Instead, wearing gloves, cover the debris with a cloth or paper towel soaked in suitable disinfectant such as a freshly made 1% (W/V) aqueous solution of sodium chlorate(I) (Hypochlorite). (Domestic bleach is in an approximately 10% solution). It should be possible to do this without bringing the face into the cloud. The debris and cloth can then be swept into a suitable container using disposable towels and the whole contaminated area disinfected. The newly introduced, disinfectant-impregnated 'Wipex' cloths may prove useful in the treatment of spillages.

Spills on clothing should be treated with a clear phenolic disinfectant (Hycolin, Clearsol, Stericol etc.) or an ampholytic surfactant (e.g. Gerrard ASAB, Harris BAS or Tego MHG) at the recommended dilution. If skin is contaminated the affected area should be carefully washed using soap and hot water. Lysol and other cresolic disinfectants are toxic and caustic and should not be used.

Choice of Organisms - all levels

The Imperial College Handbook 'Biohazard' provides a fairly comprehensive list of dangerous pathogens.(4) The Howie Report gave lists of organisms and materials, designated Categories A, B1 and B2 which require special precautions and facilities even when handled by professional microbiologists.(10) It should be obvious that none of these organisms or materials should ever be used in schools. It is also inadvisable to use any organism or strain which has been reported as causing any infection in man and where the hazards are so considerable they cannot be effectively controlled.(8)

A list is given below of selected micro-organisms, drawn from recent science teaching projects, which present minimum risk given good practice. Caution must be exercised in the use of such a list. There is often considerable delay in reports of pathogenicity reaching educational journals. In any case, there are arguments against basing a microbiology safety policy solely on categories of hazard. So-called 'harmless' saprophytes have become opportunist pathogens, often in immunosuppressed or allergic individuals. Since 'not-proven' is the firmest verdict that can usually be reached, it is best to assume guilt.

In considering any list of organisms, it is essential that cultures are obtained from a reputable supplier or Culture Collection to ensure correct identification and non-contamination by other, possibly pathogenic, organisms. For example one should ensure that cultures supplied as Staphylococcus epidermidis are a true S. epidermidis and not a white Staphylococcus aureus. Some strains of Escherichia coli are pathogenic but responsible suppliers take care that only the non-pathogenic strains are supplied. It should also be appreciated, after previous comments, that almost any procedure culturing bacteria on ordinary growth media at about pH7 may also concentrate pathogens from the environment. The Microbiological Consultative Committee have strongly recommended that one way to overcome this problem is to concentrate, at school level, on microbiology using growth media at low pH (pH4-5) and organisms such as lactic acid bacilli, acetobacter and acidophilic yeasts which are unlikely to present a significant hazard. Such an approach has much to recommend it. One could also add other organisms that require somewhat special conditions such as

a saline medium and/or have optimum incubation temperatures considerably lower than 37°C (e.g. Photobacterium phosphoreum), and yet others such as Vibrio natriegens (Beneckea natriegens), which is very sensitive to heat and disinfectants.(11) Such characteristics can make certain organisms particularly suitable for use with inexperienced students.

List of Organisms: Micro-organisms suitable for experiments in school laboratories. The listed bacteria and fungi are those quoted in the list recently circulated by the Microbiological Consultative Committee. Organisms marked thus * are regarded as particularly suitable.

Bacteria

* Acetobacter aceti
* Agrobacterium tumefaciens
  Bacillus subtilis
* Chromatium sp.
  Chromobacterium lividum
  Erwinia carotovora
  (E. atroseptica)
  Escherichia coli
* Lactobacillus casii
* Lactobacillus bulgaricus
  Micrococcus luteus
  (Sarcina luteus)
* Photobacterium phosphoreum
  Pseudomonas fluorescens
  Rhizobium leguminosarum
* Rhodopseudomonas palustris
  Rhodospirillum rubrum
  Spirillum serpens
  Staphylococcus albus
  Staphylococcus epidermidis
  Streptococcus lactis
  Streptomyces griseus
  Streptomyces scabies
  Vibrio natriegens
  (Beneckea natriegens)

Fungi

* Agaricus bisporus
  Armillaria mellea
  Botrytis cinerea
  Botrytis fabae
  Chaetomium globosum
  Coprinus lagopus
  Fusarium oxysporum
  Fusarium solani
  (Rhizoctonia solani)
  Helminthosporium avenae
  Mucor hiemalis
  Mucor mucedo
  Myrothecium verucaria
  Penicillium roqueforti
  Phycomyces blakesleanus
  Phytophthora infestans
  Physalospora obtusata
  Pythium debaryanum
  Rhizopus sexualis
  Rhizopus stolonifer
  Rhytisma acerinum
* Saccharomyces cereviseae
  Saccharomyces ellipsoides
  Saprolegnia litoralis
  Schizosaccharomyces pombe
  Schlerotina fructigena
  Sordaria fimicola

Notes It will be noted that this list differs from that given in Education Pamphlet No. 61.(8) Aspergillus species have been removed because old cultures could be a hazard to children who are asthmatic and whose condition might be unknown to the teacher. Also, Aspergillus niger is known to cause ear infections. Penicillium chrysogenum and Penicillium notatum have also been removed as they both produce mycotoxins and there is the general problem of penicillin sensitivity in some individuals. Penicillium roqueforti (used in blue veined cheeses) has been inserted as a safer substitute. It is important to handle fungal cultures just as carefully as bacterial ones and to avoid breathing in appreciable quantities of fungal spores. In addition to the slight risk of infections a range of allergenic reactions are known to occur.

Viruses (for sixth year work)

Bacteriophage (T type, host E. coli)
Cucumber mosiac virus
Potato virus X
Potato virus Y (a)
Tobacco mosaic virus
Turnip mosaic virus

(a) Not the virulent strain of virus Y causing veinal necroses as well as the usual symptom of distorted leaf margins. The Department of Agriculture and Fisheries are trying to control the spread of this strain and material infected with it should not be used.

Algae, Protozoa, Lichens, Slime moulds

Though some protozoa are known to be pathogenic the species quoted for experimental work in recent science projects, together with the species of algae, lichens and slime moulds quoted, are acceptable for use in schools.

School work can be more than adequately covered by using only the listed organisms. Teachers wishing to use other organisms, e.g. for sixth year projects should seek competent advice. We are prepared to obtain such advice on behalf of teachers through contacts with the Microbiological Consultative Committee, Government Departments and Advisory Groups. The list of suitable organisms will be kept under review and amendments issued where necessary.

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Appendix

Sterilisation by Heat

It is prudent to check occasionally the efficacy of sterilising procedures used to prepare media and for disposing of used cultures. Rubber seals on autoclaves can become inefficient. The recommended pressure of $103 kNm^{-2}$ ($15 lbin^{-2}$) may not be maintained and the required temperatures may not be achieved. Equally ovens may not be reaching the temperatures shown on thermostat settings or calibration charts.

It is worth considering just what is required in order to sterilise media for use or materials for disposal. Bacteria which the sterilising process is to destroy can be regarded as existing in two forms - vegetative forms and spores. All bacteria occur as vegetative cells, but only some of them form spores. These spores which are in a resting state, are very resistant to destruction by heat and other agents. The type of bacteria supplied for use in schools are usually non-spore forming. These vegetative forms have a thermal death point of about 60°C. They will all be destroyed after 30 mins. at this temperature and much more rapidly in boiling water. However cultures may well be contaminated with spore forming organisms, some of which may be potential pathogens. The chances of this occurring are substantially increased if cultures have been inoculated with a natural material such as soil.

Destruction of spores in the dry state requires prolonged exposure to a very high temperature (typically at least 1 hour at 160°C). They are more easily destroyed by steam, but this must be applied for a considerable time at a suitable temperature, which means at an increased pressure, in order to ensure complete destruction.

Elaborate studies over the years have yielded comprehensive information on the thermal destruction of spores of pathogenic bacteria. These studies have shown that the process has a very definite time/temperature dependence. This is explained by a number of factors not the least of which is the variation in susceptibility within

a species, or even within a particular strain. Therefore it is essential that the TIME of exposure to the steam be adequate for the TEMPERATURE, which in turn depends upon the PRESSURE employed, and that a proper MARGIN OF SAFETY is left when fixing a standard.(12)

With these factors in mind the Medical Research Council has published the following times for treatment by steam at the given temperatures:

(a) 3 minutes at 134°C (206kNm^{-2} or 30lbin^{-2});
(b) 10 minutes at 126°C (137kNm^{-2} or 20lbin^{-2});
(c) 15 minutes at 121°C (103kNm^{-2} or 15lbin^{-2}).

It is standard (c) which is most likely to be applicable in the school situation and which must be achieved or surpassed when sterilising materials for use or disposal. How then can we ensure that this is done? The first requirement is for a proper use of the pressure cooker, or autoclave, and the second for a test that will indicate whether the necessary conditions for sterilisation have been achieved. In the use of a pressure cooker the following points are particularly important; some may seem to be stating the obvious but we think that they will stand re-emphasis.

1. All the weights, or parts of a spring controlled assembly necessary to give the full 103kNm^{-2} (15lbin^{-2}) loading should be present.
2. If the loading is provided by the common type of device where a set of weights has a tapered shaft which seats in the steam outlet, ensure that it is not worn and that the shaft and seating are undamaged.
3. Inspect the sealing ring and the rubber of the safety valve. Ensure that they are not perished and that the latter is properly seated.
4. If the cooker is an old one inspect it carefully for signs of corrosion, especially pitting of the walls or bottom. This can reduce the wall thickness and make the cooker potentially hazardous.
5. The correct volume of water should be used. For most cookers this will be approximately 1 litre for 15-20 minute sterilising time. Note that this is a greater volume than that recommended by the manufacturer when vegetables etc. are being cooked, because the sterilising time is considerably longer than the usual recommended cooking times. Should the cooker be boiled dry it may become badly distorted. In the unlikely event of a failure of the safety valve the consequences would be more serious.
6. It is essential not to overload the cooker. Enough space should be left between items to allow the steam to penetrate and the air to be displaced.
7. If autoclavable disposal bags, or roasting bags, are used they should only be two-thirds filled and closed with a wire-cored paper tie. They should not be sealed. Similarly any screw caps on jars or bottles should be loosened slightly. If air is trapped it may burst containers in being prevented from escaping. However after autoclaving, disposal bags are sealed before incineration or placing in the refuse bin.
8. When placing the lid in position take care not to nip or distort the seal. Some lids will only fit properly in one position and usually this is indicated by engraved marks on the lid and lower part of the cooker.

9. For heating the cooker, a gas ring or electric hotplate is much safer than the commonly used two tripods/two bunsens arrangement.

10. Bring the water to the boil and allow a vigorous escape of steam for 2 to 3 minutes before placing the weight in position. This is to allow air to be expelled from the cooker.

11. Allow the cooker to come up to pressure so that steam escapes audibly from under the weight. Timing should start at this point and not before. It may be necessary to give 20-30 mins. rather than the recommended 15, especially if 'non-standard' materials are being autoclaved prior to disposal. Slightly reduce the heat if the escape of steam becomes too vigorous. However the weight should be 'dancing' during the whole of the sterilising period.

12. Do not cool the cooker rapidly under the tap but leave it to cool and depressurise for at least 10 minutes.

Performance testing of autoclaves, pressure cookers and ovens.

(a) Biological methods are the surest but involve a delay whilst samples are incubated. The periodic use of spore strips of Bacillus stearothermophilus is recommended as a check on the cheaper, less time-consuming chemical indicator methods.

(b) Non-biological methods; use chemical thermal indicators. The use of steriliser control tubes with a traffic light sequence is recommended as a routine method. 'Brownes' tubes made by Albert Browne are relatively inexpensive and are used by many hospitals and microbiology laboratories. Type 1 and type 3 tubes are the ones relevant to schools. Type 1 (black spot) is used for ordinary vertical autoclaves and turns green after 16 minutes at 120°C. Type 3 (green spot) is for ovens and sterilisation by 'dry heat', turning green after 60 minutes at 160°C. At about 3p per test they give a relatively inexpensive but useful indication of autoclave or oven performance. Similar but more expensive thermal indicator systems are marketed by other firms.

Autoclave indicator tape is only meant to differentiate processed from unprocessed items and should not be used in an attempt to measure performance. This statement is based on advice from the manufacturers - 3M (UK) - and is contrary to the advice given recently by several authors.

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Trade News

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Unilab, Clarendon Road, Blackburn, Lancs BB1 9TA.

<u>Sources of reliably identified cultures:</u>

Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey TW9 3AF (fungi).

Culture Collection of Algae and Protozoa, 36 Storey's Way, Cambridge CB3 ODT (algae and protozoa).

Food Research Institute, Colney Lane, Norwich NOR 70F (yeasts).

Gerrard Biological Centre (see above - algae, bacteria, fungi, protozoa and yeasts).

Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon BS24 9BJ (as Gerrard).

Torry Research Station, P.O. Box 31, 135 Abbey Road, Aberdeen AB9 8DG (bacteria).