MiSAC*methods* 2
PRODUCED BY THE *MICROBIOLOGY IN SCHOOLS ADVISORY COMMITTEE*

Sourcing, maintaining and using cultures

Sourcing suitable cultures

- Microorganisms on the list approved for use in schools and colleges present minimum risk, given observance of good microbiological laboratory practice (GMLP); see www.misac.org.uk, *Health & safety: Suitable and unsuitable microorganisms*.
- The list is not definitive; other organisms may be used if competent advice is taken.
- The current version of the list should be consulted because recommendations are altered from time to time in accordance with changes from experience and after risk assessment.
- Cultures should be obtained from a reputable specialist school supplier. Isolation of cultures from the environment may be conducted if appropriate to the level of work, i.e. L1, L2 or L3 (see www.misac.org.uk, *Health & safety: Safety guidelines* and *Useful Links: Suppliers*).

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

Maintaining and using cultures of bacteria and fungi

Cultures of bacteria and fungi are provided by school suppliers on slopes of agar culture media. They should be subcultured on the same or a similar medium which may be in either agar or broth, (ie, liquid form). Instead of purchasing a new culture each time it is needed, you might consider maintaining a laboratory collection of stock cultures. However, to achieve success, maintenance of your own stock cultures must be well organised, with attention to detail. You must be prepared to transfer cultures to fresh medium at *not more than 3-monthly intervals* to maintain viability but shorter intervals might be necessary, eg, for lactic acid bacteria such as *Lactobacillus.* Survival of moulds may be longer because of the tolerance of their spores.

Most of the cultures suitable for school use are relatively easy to maintain by subculturing on the medium on which they are provided. For bacteria, the medium is usually nutrient agar but lactic acid bacteria are obligate fermenters and therefore need special media that contain a fermentable substrate. Thus *Streptococcus* and *Leuconostoc* are cultured on a special medium that contains glucose and yeast extract, eg, GYLA (glucose-yeast extract-'Lemco'-agar) medium; *Lactobacillus* grows best on MRS medium. (Note: 'Lemco' is a meat extract; MRS refers to the names of the originators of the medium.) For fungi, the medium is usually either malt extract agar, potato dextrose agar (PDA), cornmeal agar, oatmeal agar or starch nutrient agar.

For storing stock cultures, the use of Universal or McCartney bottles fitted with screw-cap closures is strongly advised because of the reduction in evaporation and drying out, and a cap is less easily displaced accidentally than a cotton wool plug. Cultures on agar slopes are preferred to broth because they provide a more concentrated inoculum for making subcultures and the first sign of contamination is much more readily noticed. Keeping stock cultures on agar plates is most unwise as these are very vulnerable to evaporation of the medium and culture contamination.

A *minimum of two* stock cultures should be prepared. One is the "permanent" stock which is opened only once for preparing a new permanent stock culture. The other culture serves as the "working" stock culture that is used for repeatedly taking subcultures for classes (see *Figure 1* which summarises the procedure).

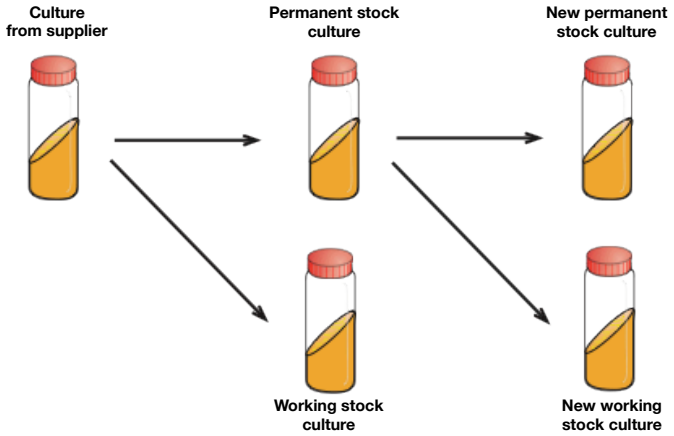

Figure 1. Maintaining stock cultures of bacteria and fungi

Incubate an inoculated medium at an appropriate temperature, eg, room temperature or 25 °C, until there is good growth which is usually 1-2 days for bacteria and yeasts, and up to a week for moulds. The screw cap should be slightly loosened during incubation to allow a good supply of oxygen, but closed securely before storage. Partial unscrewing of the cap is important for providing an adequate amount of oxygen for the growth of strict aerobes, eg, all moulds and the bacteria *Pseudomonas fluorescens* and *Bacillus subtilis*, but it is also useful for facultative aerobes, eg, *Escherichia coli* and yeasts. The temporary loosening of the screw cap of a bottle is also necessary during the sterilisation stage of media preparation in order to ensure that the contents of the bottle are exposed to the high temperature generated in the autoclave by saturated steam under pressure, eg, 121°C at 15 lb psi (103 kPa).

As soon as there is adequate growth, store the cultures either in a refrigerator or at room temperature in a drawer or cupboard for security and to avoid direct sunlight. There need be no concern about loss of viability if a refrigerator malfunctions and the temperature rises to room temperature but be vigilant for signs of contamination.

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Checking cultures of bacteria and fungi for contamination

Evidence for a culture being pure or otherwise is given by the appearance of colonies on a streak plate (see *Figures 2 & 3*) and of cells in a stained microscopical preparation, preferably using Gram's method (see *Figure 4*). There should be uniformity of colony and cell form, and consistency with the appearance of the original culture. It is sensible to check the purity of the working stock culture from time to time, and of the permanent stock when preparing new permanent and working stock cultures.

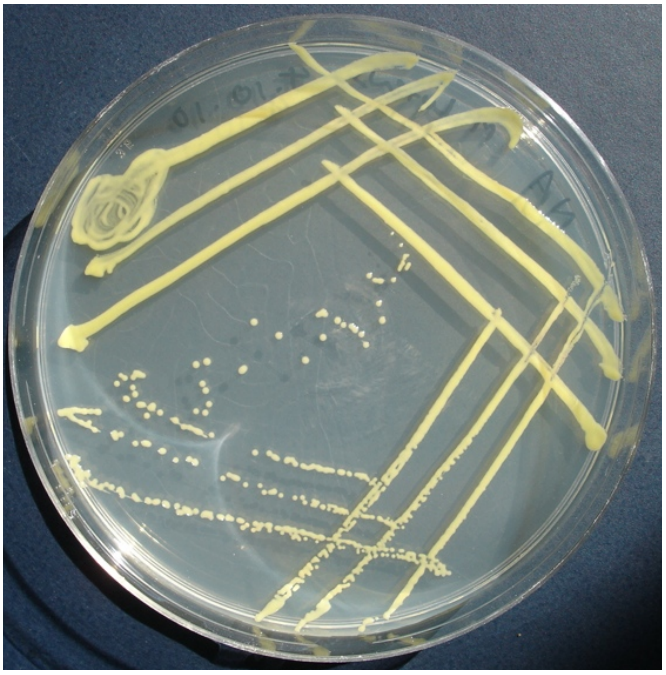

Figure 2. Streak plate of a pure culture of bacteria showing identical colony forms Image: J Schollar CC BY-SA 3.0

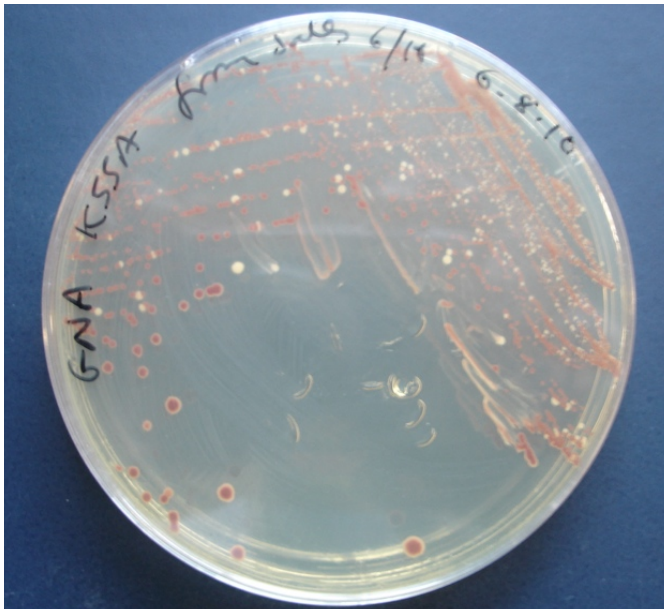

Figure 3. Streak plate of a mixed culture of yeast showing different colony forms Image: J Schollar CC BY-SA 3.0

If a culture becomes contaminated, do ***not*** try to remedy the situation by preparing a streak plate from the contaminated culture and taking an inoculum from a single colony. This is because of the possibility of (i), not being able to distinguish between the colony forms of the contaminant and the original culture and (ii), culturing a variant of the original culture that does not behave as the original culture did. Instead, go back to the unopened permanent stock culture - that is what it is for!

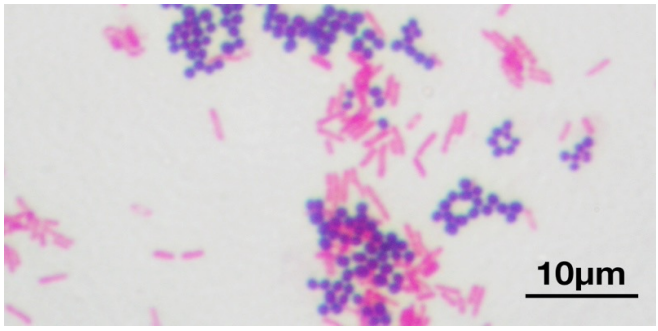

Figure 4. Smear of a mixed culture of bacteria stained by Gram's method Image: Y tambe CC BY-SA 3.0

Maintaining and using cultures of protozoa and algae

Protozoa are supplied as liquid cultures or in dried form; algae may be in liquid culture or on an agar medium but the former is usually preferable. The cultures are usually intended for use in the form provided by the supplier without the need to be subcultured. Therefore, record the date of receipt and maintain the culture until use, according to the recommendations provided, including loosening the cap to allow access of air and keeping the culture chilled until needed. It is important to note that not all protists remain viable to the same extent in laboratory culture. For example, many algae remain viable for several weeks if kept cool and illuminated but others, eg, *Spirogyra* and *Volvox*, might die after a week or two.

Preparing cultures for class use

Unlike in other disciplines where materials for practical classes such as solutions of chemicals or electrical circuits can be taken from the store ready for immediate use, microbial cultures must be prepared well in advance to ensure that they are physiologically active at the time of the class. Otherwise, the outcome might not be as expected and the educational experience will be either diminished or lost.

Bacteria and yeasts. Transfer cultures sequentially several times in advance to ensure that they are growing well and are presented to the class as young, actively-growing cultures. This is achieved by taking an inoculum from the working stock culture into preferably a broth medium in a Universal bottle or a test tube. As soon as good growth has developed, inoculate from it into fresh medium and repeat the sequence several times (see *Figure 5*).

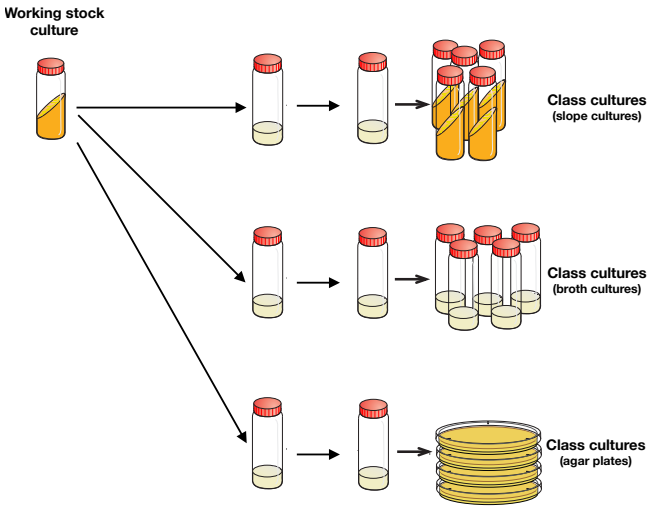

Figure 5. Preparation of cultures for class use

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Figure 6. Transferring cultures in test tubes. Note: holding two test tubes in one hand, as shown, is a valuable manipulative skill for a student to learn and saves time when a large number of transfers are to be made.

Progress of growth can be followed by observation for turbidity (cloudiness) by the naked eye. Growth should be well distributed to provide a suitably concentrated inoculum by gently tapping the side of a tube of culture against either the palm of the other hand or horizontally-held closed fingers. The bacterium *Bacillus subtilis* presents a slight problem because its growth in a liquid medium is characteristically concentrated at the surface in the form of a tough skin (known as a pellicle) which leaves clear medium beneath containing very few cells. In this case it is necessary to persevere until the skin is broken into small flakes that will be included in the inoculum.

The main points in achieving good growth are as follows.

- Use an adequate amount of inoculum, eg, 1 loopful to 5-10 ml of culture medium or 5% (v/v) to a larger volume.
- Choose a suitable culture medium and incubation temperature (see www.misac.org.uk, *Health & safety: Safety guidelines*).
- Provide adequate aeration when growing a large volume of liquid culture (ie, more than about 20 ml) of a strictly aerobic organism such as the bacteria *Pseudomonas fluorescens* and *Bacillus subtilis*, by using either a shallow layer of medium in a static conical flask (eg, 50 ml in a 250 ml flask) or a larger volume of medium aerated by agitation by a magnetic stirrer.

Inoculating a large number of cultures of bacteria or yeasts for a class by using a wire loop is time consuming. Alternatively, a more-efficient procedure is to take a sample of culture using a Pasteur dropping pipette. If slope cultures are needed, a straight line of growth is produced by drawing the tip of the pipette along the surface of the agar medium.

Moulds. Moulds are usually grown on the surface of an agar medium because their characteristic growth in liquid media is as a dense surface mat which is not convenient to use. Moulds grow more slowly than bacteria and require an incubation period of a few days to a week at room temperature or 25 °C.

Often the inoculum taken from the surface of an agar medium is a portion of the mycelium, usually together with a portion of the culture medium. A convenient instrument is either a wire loop or a straight wire with the last few millimetres bent at a right angle. When an agar plate with a mould inoculated at the centre is required, it is easy, inadvertently, also to inoculate other areas of the medium with portions of mould, usually spores, that fall from the loop or wire. This can be avoided by placing the closed Petri dish on the working surface, lid downwards, lifting the base (containing the medium) vertically above the lid and using a bent wire to introduce the inoculum upwards to the centre of the downwards-facing medium surface.

Protozoa and algae. When supplied in liquid culture, many of these microorganisms remain alive for several weeks if maintained according to the recommendations provided. Such cultures are ideal for microscopical examination, especially when observed within a few days of receipt. After further storage, however, some features may be lost, eg, protoplasmic streaming and pseudopodia formation in amoebae.

There are also interesting physiological investigations with algae for which larger volumes of culture are needed. It is then necessary to ensure that an adequate stock of the appropriate culture medium has been prepared. The volume of inoculum should be about 10% (v/v) of the culture medium and at least a week allowed for growth at room temperature with a day-time maximum of 25 °C. Cultures of algae require illumination by either daylight or artificial light.

Other sources of information

Health and safety. www.misac.org.uk. *Health & safety.*

Topics in Safety (2018) 4th ed. Association for Science Education.

Suppliers of cultures and other items. www.misac.org.uk, *Useful links: Links and publications, Suppliers.*

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