MICROBIOLOGY:
Microbiology Basics

For the workshop today we will be focusing on three basic areas of knowledge for working in the discipline of microbiology; preparation of media, the actual working phase of the activity and then clean-up or disposal. These notes are intended to prompt your memory of our discussions and are not intended to be a comprehensive document.

Sterilisation and sterile techniques are very important for the basics of microbiology. When you prepare media for bacterial or fungal growth you need to know that the media is clean and sterile, and that the only introduction to it is the one that you are about to make. For this reason, we sterilise our media after it has been made, and we use aseptic techniques when we introduce specific micro-organisms.

Selecting media

There are many recipes available for making media. Our experiences at Southern Biological are with the commercial products from reputable Media manufacturers. If you are using a recipe from elsewhere, we recommend that you conduct a trial to ensure that the growth is as you require. There are many media within the Oxoid range, but we will focus on the basics.

- Nutrient broth and Nutrient Agar are used for culturing bacteria
- Malt Extract Agar is used when growing fungi
- Plain agar contains no nutrients and will not support the growth of either bacteria or fungi. I mention this because colleagues will possibly refer to “agar plates”. If they wish to grow bacteria, they really meant to say “nutrient agar plates”, as the word “agar” alone can lead to misunderstandings.

Preparing media

Whether you are making an agar or a broth, follow the instructions of the manufacturer. The general approach is that you paste the powder using a small amount of room temperature distilled water and then while stirring, add the balance of the distilled water. Bring the solution to the boil while stirring and then decant into containers that are suitable for sterilisation. If your medium is an agar, it is very important that you boil for a minimum of 3 minutes to allow the agar component to swell and take the right form. When you place your container into the sterilisation unit, get into the habit of tightening the lid and then ¼ turn releasing the lid. The ¼ turn release allows steam to displace air in the container as the pressure increases. Once sterilisation has been completed, tighten the lids, then label and date the bottle. Sealed bottles will keep for approximately 3 months.

Sterilisation

The Australian Standard AS2243.3 defines sterilising conditions as 121ºC at 15 psi for 15 minutes. When you use a hotplate or a microwave the media experiences atmospheric pressure and therefore the temperature is no more than 100ºC.

Using media

Broths are used as a liquid, and agar media is used as either a plate or as a slope. Your bulk, sterile broth media can be divided into smaller volumes using aseptic technique. The method using a flame will be demonstrated and practised during the workshop. This can be done with the working, sterile broth at room temperature. The bulk broth has been sterilised, so the receiving container also needs to be sterile.

To transfer your bulk sterile agar media into plates, you will need to either use it straight after sterilisation while it is still warm or you will need to use a water bath to "melt" the agar to a pourable state. If you are using a water bath, ¼ open the lid of the media bottle and place it in a water bath at 100ºC until the media is liquid. Cool the bath to approximately 70ºC. Close your work area and prepare the working bench by wiping with 70% alcohol or QAC solution (or equivalent) and then aseptically pour the plates, or make the slopes. Leave plates and slopes until fully set. When plates are set, date and store them in the fridge, with plates sitting "upside down" on their lids. When slopes are set, tighten the lids and store in the fridge.

Micro organism Risk Groups

Bacteria and Fungi are living organisms and therefore do not have an MSDS. They are defined by a "Risk Group". The Australian Standard AS2243.3 defines the risk groups for each organism. All micro-organisms that are sold by Southern Biological are classified as Risk Group 1, which are considered to be non-pathogenic and therefore safe to use when handled correctly.

Working technique and work area

If you haven't done aseptic technique for some time, practise before you start. It is a good idea to get your technique and your hand movements back in practice for the task at hand. When you are working aseptically it is best if you adopt the following procedures:

- Have your room sealed from air movement (and people movement)
- Gather together all your equipment
- Wipe your work area with 70% alcohol or another suitable surface sanitiser.
- Have a flame operational
- Clean your hands
- Don't talk while working
- When containers or plates are open, only open them as far as is necessary
- When the work is complete, wipe your work area with 70% alcohol or another suitable surface sanitiser.

Making lawns

A lawn is basically a complete covering of microbial growth over a plate. This can be most easily created via a sterile swab or sterile spreader. It is very important that there is not excess liquid, so we recommend that the swab be dipped into the bacterial broth and then pressed carefully against the side of the vessel to remove excess liquid before the swab is used on the plate. Using the same swab cover the plate in three directions, 0°, 90° and then 45°. This will be demonstrated during the workshop. Alternatively, use a sterile pipette to place 3 small drops of bacterial broth onto the plate and then using a dry sterile swab, or sterile spreader, distribute the liquid over the surface using the same three directional action. Regardless of your technique it is important to keep excess liquid to a minimum. Excess liquid creates a surface cover of liquid and vapour. Both can cause complications during experiments, particularly if antibiotic discs are being employed.

Using Antibiotic discs and Mastrings

Antibiotic discs or Mastrings are placed into the plates about 30mins after the lawn has been created. Use sterile forceps to make the placement and gently press to ensure good contact. Incubate the plate, upside down. The zones of resistance can be observed after incubation.

Incubation

- Bacteria at 33-37ºC for 24-48 hours.
- Fungi at room temperature for several days.

Use of experimental controls
We receive a number of phone calls each year where clients advise us that their bacteria are not growing. We have found that when this comment is linked to antibiotic work, the problem is not usually the bacteria, nor the media, it is most often linked to the amount of moisture left on the plate from when the plate was poured, or the amount used when the lawn was created. When there is excess moisture, the moisture layer on the plate surface can pick up the antibiotics in the discs and distribute the antibiotics all over the plate surface. If the bacteria are susceptible to the antibiotic, the growth of the whole plate will be suppressed. This makes it look as though the bacteria have not grown. Poor technique leads to the wrong conclusion. We encourage all our clients to use a "control" during experimentation and in this particular case the control would be a lawn plate that has no antibiotics on it. Growth on the control plate will confirm that the bacterium is in good condition and that the results on the test plate are most likely the result of the antibiotic being spread too widely, which is possibly caused by the techniques employed.

Subculturing
If you have a pure culture and you wish to increase the volume, all that is required is that you inoculate the culture into the next sterile volume. Aseptic technique using either a loop or a "transfer tip of solution" is acceptable. After incubation the larger volume will be populated.

Making single colonies
It probably won't be necessary for you to have to make your own single colony plates, but in order to show you how it is done, we will demonstrate how they are made and inspected. The purpose of streaking a plate for single colonies is to ensure a pure culture is being propagated.

Seal plates that students have inoculated
When students have completed the experimental task on a plate, use two pieces of "sticky tape" to close the plate. Doing this prior to incubation means that students are not tempted to open the plate after incubation. This becomes particularly important if the experiment has involved swabbing surfaces rather than culturing from a known source. Unknown bacteria have the possibility of being pathogenic and therefore it's important that the lids are not taken off the plates.

Petrifilm
Petrifilm are dehydrated plates from the company 3M. Each plate has a medium on it that needs to be rehydrated prior to use. The plates tend to be specific for a task, or for identification of specific bacteria. At Southern Biological we sell four plates. The plates require a specific inoculation volume of 1mL. With the aid of a "spreader" the liquid is distributed over a specific area of 20 square cm. The plate is then placed into a press seal bag, incubated and read after incubation.

Clean-up and Disposal
Everything that has been used will need to be sterilised at the end of the process. Sterilisation will require the surface to be exposed so if plates are sealed, open the seal, if lids are tight, loosen them and if you are using autoclave bags, either open them or cut slits in the upper area so that the steam can penetrate. Autoclave or sterilise at 121ºC and 15 psi for at least 15 minutes.