SECTION G

STERILE TECHNIQUE AT HOME OR AT SCHOOL

A. Preparing agar plates

Equipment
- Domestic pressure cooker
- Agar and other ingredients listed in Recipes section
- Disposable plastic petri dishes
- Damp tea towel
- Fairly small, clean, draught free room
- Methylated spirit
- Spirit lamp or Bunsen burner.

1. Agar medium can be prepared in small quantities in medicine bottles or soft drink bottles with metal caps.

2. Weigh (or measure by equivalent volume) the required amount of agar into the bottle and add the water. Never fill bottles more than 2/3 full and always leave caps loosely on during sterilization. Make sure there is 2 cm clearance between the top of the bottles and lid of the pressure cooker.

3. Put bottles into pressure cooker with about 4 cm of water. Put on lid and heat till steam is hissing strongly from the vent. Add the heavy weight (15 lb or 100 K Pa) and continue heating until the steam is escaping from the valve which indicates pressure has been reached. Turn down heat to keep it snorting gently and sterilise for 20 minutes. Turn off heat and allow to cool slowly.

4. Pour agar into plates in a small draught free room. Wipe down a table with methylated spirit then place a clean damp tea towel on the table and arrange the sterile petri dishes on it.

5. Remove the cap from the bottle of agar and flame the neck then pour 15-20 mL into each plate, trying to avoid removing the lids completely. Keep the bottle held at about 45° to prevent drips running up and down. Leave petri dish lids slightly ajar till the steam stops condensing on the lids then shut them. Leave plates till agar has set then wrap dishes in “Gladwrap” until needed.

6. Wash out bottles immediately after use. Never pour undiluted agar down the sink – it sets and blocks the drains. Always wash well diluted agar away with plenty of hot water.

B. Setting up cultures

To do this you may need to use sterile forceps, Pasteur pipettes or sterile filter papers etc. Things like these can be sterilized by enclosing them in a screw top jar and sterilizing in a pressure cooker as for the agar.

C. Subculturing

Equipment As for A. but you will also need a wire inoculating loop and/or a mounted needle.

1. Working on a swabbed down table and a wet tea towel as before, heat the mounted needle or inoculating loop until red hot and leave to cool (it takes quite a while – have an initial test run using your fingers to see when it is cool).

2. When cool, transfer to a small portion of the fungal or bacterial colony from one petri dish to a fresh one taking care not to completely remove the lids of the dishes. Re-sterilize inoculating wire.

D. Recipes

1. Malt extract Agar

Malt extract	20 g (sticky malt from brewing shops)
agar	20 g
water	1 litre

Source: *Biology Projects for High School Students*, by Prof. Jennifer McComb, School of Environmental and Life Sciences, Murdoch University, Western Australia. Used with permission.
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2. Potato dextrose Agar

potato	200 g
dextrose	20 g
agar	20 g
water	1 litre

a) Scrub potatoes but do not peel, cut into 10 cm cubes. Weigh out 200 g.

b) Rinse rapidly in running water then place in 1 litre water and boil until very soft (1 hour).

c) Mash roughly with remaining water and strain through a sieve. You need the liquid and a little sediment. Add agar and dextrose, make up to 1 litre, with water, sterilize.

3. Tap water or plain Agar

agar	15 g
water	1 litre

4. Rabbit dung agar (for dung fungi – Project 1-1)

Sterilize rabbit pellets, in a screw top jar. Add six to each petri dish, cover with tap water agar.

5. Cornmeal Agar (for nematode trapping fungi – Project 1-4)

maize-meal (Polenta)	20 g
tap water	1 litre
agar	20 g

a) Mix water and maize-meal and heat to 70 C for 1 hour. Allow to stand and pour off the clear solution. Make up to 1 litre.

b) Add agar and autoclave.

General Note - If you cannot weigh out ingredients at home, weigh them out at school in test tubes and carefully mark the levels on the tubes. Then for later work at home you can use these marked tubes and use equivalent volumes rather than weighed amounts.

Disposal - sterilize used cultures in glass petri dishes using a pressure cooker, or incinerate cultures in disposable plastic petri dishes.