CCAP CULTURE MEDIA INFORMATION SHEET

CHEMICALS:

- Media should be made with the highest quality chemicals available.
- Check the quality with the manufacturers, websites and company catalogues will provide this information.

EQUIPMENT:

- Minimal equipment required: glassware, plastic-ware, analytical balance, top-loading balance, pH meter, hot-plate magnetic stirrer, and magnetic stirrer. General PPE.
- Regular equipment maintenance, calibration and record keeping are good practices.
- An autoclave is usually essential for sterilization. As well as filtration equipment such as vacuum source and filter syringe.
- A laboratory dishwasher that uses deionized water is recommended for stubborn dirt.
- A refrigerator with a freezer compartment for maintaining stock solutions and culture media.
- When siphoning water from one vessel to another, be aware that using latex tubing can render the water toxic for phytoplankton growth. Silicone tubing has been found to be completely safe to use.
- When an autoclave is not available, media can be sterilised using a pressure cooker, or in the microwave (loosely covered to reduce loss through evaporation).

FRESHWATER:

- For freshwater culture media, water of the right quality is obtained from double-distilling apparatus or from a deionized water system with carbon and membrane filters. The level of quality is determined by the sensitivity of the organism and the application.
- If you don't have access to deionized water, bottled mineral water can be used.

SEAWATER:

- For marine culture media, it is recommended for seawater to be collected offshore, as open ocean water is low in nutrients and trace metals, which can be added in the required amounts. In addition, the seawater contains less sediment and possibly less phytoplankton, making it easier to filter. Normally, seawater should be filtered to 0.45µm with membrane filters, or with glass fiber filters, using GF/F filter with a pore size of 0.7µm recommended. Filtered seawater can be stored in either glass or plastic carboys, kept cold (preferably refrigerated) and in the dark (or covered with black plastic).
- After autoclaving filtered seawater, leave the media for 24 hours to equilibrate, so that the gases such as CO_2 are allowed to diffuse into the medium.
- To help avoid precipitation, cooling the seawater quickly after autoclaving by standing it in cold water in a sink or sterilizing smaller volumes at a time is also known to help.

ARTIFICIAL SEAWATER:

- Most marine culture media are made with seawater, including ASW, despite being already named Artificial Seawater. In the absence of filtered natural seawater, or if you don't wish to use natural seawater, artificial seawater can be made using sea or ocean salts, for example the brands Instant Ocean or Tropic Marin, others are available. Sea salts contain a range of micronutrients and elements. Take care with "Aquarium Salt" which is sometimes simply NaCl.
- Artificial seawater can be made to any specific salinity.

SOIL/SOIL EXTRACTS:

- Liquid soil extract or solid particulate soil is used for culturing some algal or protozoan species. The recommended sources for good soils are gardens or greenhouses where the soil has not been exposed to chemicals, undisturbed deciduous forests, and grasslands that have not been tilled or grazed.
- For certain organisms, it might be possible to obtain soil near the source of water where they grow naturally. However, the soil must be taken above the water level, because underwater lake and pond sediments are often anoxic and contain toxic materials.
- After removal of any obvious extraneous material, the soil should be dried at a low temperature (<60°C) until it can be easily crumbled into a fine dust. Then passed through a sieve to remove any large particles and stored in a dry environment.
- To prepare a soil, add 1 part soil to 2 parts deionized water and autoclave for about 2 hours. Allow to settle, and filter the liquid. The extract liquid should then be autoclaved again to establish a sterile solution. It should be tightly capped and stored at 4°C.

STOCK SOLUTIONS

- Stock solutions are useful for the following reasons; repeated individual weighing of chemicals is time-consuming and errors may occur. While stock solutions are made occasionally, and once made, it provides an easy and consistent source.
- Stock solutions are made with deionized water and should be stored in tightly sealed glassware or plastic-ware to avoid evaporation which can lead to alteration of concentration.
- Media are generally made up of 3 components: ***macronutrients, trace elements*** and ***vitamins.***

Macronutrients- They should be prepared at a concentration of 100- to 1000- fold of the final concentration, so as to use 10ml and 1ml, per 1000ml of medium. Phosphate stock solutions should never be stored in polyethylene bottles (the phosphate ions are absorbed onto the polyethylene). Silicate stock solutions should be stored in non-vitreous material such as Teflon-lined, polyethylene, or polycarbonate (due to the dissolution of silicic acid from glass).

Trace Elements- Are usually prepared as separate stock solutions or mixed stock solutions. Na_2EDTA (di-sodium ethylemediaminetetraacidic acid) is used as a chelator. It should be completely dissolved first, followed by the addition of the metals. Trace metals can be prepared as 'primary' stocks of high concentrations to make the weighing of amounts easier. These stocks are used to make the 'working' solutions from which the final media is prepared. Because some solutions are kept for long periods of time, it is recommended to mark the liquid level and keep cold and tightly sealed with parafilm to reduce the amount of evaporation which can alter the concentration.

Vitamins- The three vitamins, Vitamin B1 (Thiamine), Vitamin B12 (Cyanocobalamin), and Vitamin H (Biotin) are usually used for the culture of algae. Vitamins are frequently autoclaved with the final medium, this does result in some decomposition, but the moieties in many instances are apparently equally effective. Strictly speaking, the vitamins should be added aseptically (*ie*. filter sterilised) to the

final medium after autoclaving. Mixed vitamin stock solutions can be dispensed into small volumes and frozen and then refrozen after each use without noticeable degradation.

OTHER USEFUL HINTS AND TIPS:

- When making media or stock solutions, use a standard preparation form where you can record all the steps first before you begin. This allows you to trace back the steps if any problems arise with your media.
- Gather all the equipment you will need together, correct pipettes and tip size (ensuring the pipettes are set on the correct volume), vessels labelled etc. A small waste container is handy for disposing of the pipette tips later, (a new tip is needed for each separate stock solution).
- To get the exact volume of deionised water or filtered/artificial seawater, measure out to the nearest 10ml and then use a pipette to distract the volume that you will be adding back in as stock solutions. (For example: 1L of Marine Z media has 3 stock solutions of 1ml each and 1 stock solution of 0.1ml per litre, measure exactly 1L of filtered seawater and then use a pipette to extract 4.1ml before adding the stocks).
- Always give stock solutions a visual check before using. Most contaminants are easy to spot and you can remake fresh stock solution.
- When adding chemical powders, it is good practice to record the Lot/Batch numbers. This allows you to be able to go back to the exact chemical used in your media if any problems occur and it also gives you the information that may be required if you need to contact the supplier with queries.

USEFUL RESOURCES:

Andersen R (2005) *Algal Culturing Techniques*. Elsevier, Amsterdam etc.

List of CCAP media recipes: https://www.ccap.ac.uk/index.php/media-recipes/