3 Preparation of the Kit

3.1 Sterilising the Filtration Apparatus

The sample cup and the filtration apparatus must be sterilised before use and re-sterilised between samples when analysing water from 2 different sources.

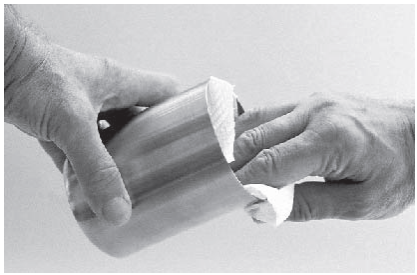
Sterilising the equipment in the field presents some practical difficulties and must be carried out using simple methods. The most appropriate is the use of methanol, which is described below. When methanol is burnt in a low oxygen atmosphere - for example, in the closed sample cup - formaldehyde gas is produced as a by-product of combustion.
Formaldehyde gas is a very effective disinfectant. Methanol is expensive to freight and requires special transport conditions. We would recommend that you first try to obtain methanol in-country from a pharmaceutical supplier, a local hospital or university laboratory. If necessary, however, methanol can be supplied by the Delagua Water Testing Ltd on request.
If methanol is not available, the filtration apparatus and sample cup can be sterilised by immersion in boiling water for 10 minutes.

Procedure for sterilising the filtration apparatus using methanol

Note: Methanol is the only alcohol suitable for sterilising the filtration apparatus; there is no substitute.

1. Carefully dry the sample cup and filtration assembly with a clean dry towel or tissue.

2. Using the plastic collar, secure the filtration funnel in the loose but not free position (see Section 5.4.4 [9]) which allows the formaldehyde gas to penetrate all areas of the filter head.



Dry the sample cup

3. Pour about 1ml (approximately 20 drops) of methanol into the sample cup.

Add 20 drops (approximately 1ml) of methanol

4. Carefully ignite the methanol in the sample cup using the cigarette lighter. Place the cup on a flat surface which will not be damaged by heat.

   Caution: Keep the mouth of the sample cup away from your face and the hole uppermost to prevent methanol running onto your hand.

Carefully ignite the methanol

5. Allow the methanol to burn for several seconds and, when almost completely burned up (ie. as the flames are dying down), place the filtration head over the sample cup and push firmly into place to form a good seal.

Replace the filtration head

6. Keep the filtration apparatus sealed for at least 15 minutes before use.

Note: It is best to sterilise the filtration apparatus immediately after each analysis and to keep the filtration apparatus in a sterile condition during transport and storage. In this way, the filtration apparatus is always ready for use.

3.2 Preparation of Culture Medium in the Laboratory

You will need the following items:

1. 38.1g of Membrane Lauryl Sulphate Broth (MLSB)(a)
2. Distilled water (b). Check that the pH of the water is between 7.0 and 7.8 using the comparator and phenol red tablets (Section 5.2)
3. Ten polypropylene bottles (60ml)
4. Measuring cylinder or graduated flask
5. Clean flask or beaker, approximately 1 litre capacity
6. Pressure cooker, steriliser or autoclave(c)
7. Heating element, stove or burner

(a) The medium is available in 38.1g, pre-weighed amounts from Delagua Water Testing Ltd
(b) See Appendix E for suggested alternative sources of water
(c) A portable steriliser kit is available from Delagua Water Testing Ltd

Method

1. Carefully wash the plastic polypropylene bottles in clean, warm water before use. If necessary, use a little detergent and then rinse well with clean water to remove all traces of the detergent.

2. Measure out 500ml of distilled water using the measuring cylinder or graduated flask. Decant approximately half of the water into the clean flask or beaker.

3. Add the 38.1g of MLSB powder to the distilled water in the clean flask or beaker and stir until the powder has dissolved. Gentle heat can be applied if the powder is slow to dissolve. Add the remaining volume of distilled water and continue stirring to thoroughly mix the broth.
   The culture medium will be a bright red colour when dissolved.

Note: MLSB is a fine, but non-hazardous powder. However, the dust may irritate the nose or upper respiratory tract if inhaled. Take care to avoid creating excess dust when handling the powder and cover the nose and mouth with a cloth or dust mask to reduce exposure. Spillages can be cleaned up using water and an absorbent cloth.

4. Pour a suitable volume of culture medium (approximately 50ml, but no less than 40ml) into each of the 10 polypropylene bottles. This provides sufficient medium in each bottle to carry out 16 tests; the maximum that can be performed in one day using the Delagua kit.

5. Replace the screw caps on the polypropylene bottles. Make sure the caps are secure but do not tighten. Leaving the caps slightly loose prevents the bottles from collapsing during sterilisation.

6. If an autoclave is available, sterilise the bottles at 121°C (equivalent to 1 bar, or 15 psi steam pressure) for 15 minutes. Tighten the caps carefully once the medium has cooled.

7. If you do not have access to an autoclave, then a household pressure cooker or portable steriliser may be used. Place the bottles in a rack inside the cooker (they may melt if placed directly on the base of the cooker), replace the lid and heat to full pressure (about 1 bar or 15psi).
   Once the cooker has reached full pressure allow steam to issue from the release valve for 5 minutes, then time the 15 minutes sterilisation cycle using a stopwatch or clock. At the end of the 15 minutes, switch off the heat and allow the cooker to cool until it is comfortable to touch. Remove the media bottles and tighten the caps.

8. Label the bottles to indicate sterilised contents and the date and batch of medium.

3.3 Preparation of Culture Medium in the Field

You will need the following items:

1. 38.1g of Membrane Lauryl Sulphate Broth (MLSB)[(a)]
2. Distilled, or clean water[(b)]
3. 10, polypropylene bottles (60ml)
4. Measuring cylinder or graduated beaker
5. Portable steriliser[(c)] or pressure cooker or cooking pot or pan

(a) The medium is available in 38.1g, pre-weighed amounts from Delagua Water Testing Ltd
(b) See Appendix E for suggested alternative sources of water
(c) A portable steriliser kit is available from Delagua Water Testing Ltd

Method

1. Carefully wash the plastic polypropylene bottles in clean, warm water before use. If necessary, use a little detergent and then rinse well with clean water to remove all traces of the detergent.
2. Use distilled water if possible. If this is not available obtain the cleanest water possible. DO NOT use water that has been treated with chlorine or any other chemical disinfectant.
3. Use the comparator and phenol red tablets in the kit to check that the pH of the water is between 7.0 and 7.8. If it is not, it will be necessary to find an alternative source of water.
4. Measure out 500ml of clean water in a beaker.
5. Add 38.1g of the MLSB powder to the 500ml of water in the beaker. Mix to dissolve the powder completely. Apply gentle heat if the powder is slow to dissolve. The culture medium will be clear with a bright red colour when dissolved.
6. Pour a suitable volume of culture medium (approximately 50ml, but no less than 40ml) into each of the 10 polypropylene bottles. This is sufficient medium in each bottle to carry out 16 tests; the maximum that can be performed in one day using the Delagua kit.
7. Replace the screw caps on the polypropylene bottles. Make sure the caps are secure but do not tighten. Leaving the caps slightly loose prevents the bottles from collapsing during sterilisation.
8. If a pressure cooker is available, sterilise the culture medium as described in Section 3.2, paragraph 7.
9. If a pressure cooker or portable steriliser is not available, the medium can be sterilised using a process called Tyndellisation. Note, this

procedure takes 3 days. Place the bottles of culture medium into a cooking pot or pan of boiling water, taking care to ensure that the bottles do not come into contact with the base of the pan (use a rack or stand) or become submerged. Boil for 20 minutes. Leave the medium to stand for 24 hours at room temperature (20-30°C) in the dark. On the following day heat the medium in boiling water for a further 20 minutes and, once again, leave to stand for 24 hours. On the third day repeat the heat treatment. The medium should now be sterile.

3.4 Storage of Culture Medium

Sterile MLSB will be stable for up to 6 months if stored in a refrigerator (between 4 and 6°C). Alternatively, the medium can be stored for up to 3 months in a cool, dark place. If the medium has been stored for several days below 6°C a deposit may form which dissolves when the medium is warmed and gently shaken. The deposit is caused by the lauryl sulphate coming out of solution.

If signs of deterioration are observed, eg. cloudiness or yellow colouration, the contents of the bottle must be discarded.

3.5 Sterilising the petri-dishes

1. Wash the dishes in a solution of mild detergent, rinse thoroughly with clean water and dry.
2. Assemble the dishes into batches of 16 in the straps.
   EITHER
3. Sterilise the petri dishes in an autoclave, steam steriliser or pressure cooker at 121°C for 15 minutes (see section 3.2 paragraphs 6 and 7).
   OR
4. Place the dishes in a conventional oven at 180°C for 30 minutes.
   OR
5. Plunge the bases and lids of the dishes into boiling water for 10 minutes. Pour away the water and assemble the dishes as they dry, but while they are still hot.
   OR
6. Add a few drops of methanol (or ethanol) to a clean cloth and wipe the inside of the lid and the base of each petri-dish. Assemble the petri-dishes and allow the alcohol to evaporate before use.

OR

7. Whenever possible, always use one of the above methods. If this is not possible, then the following method can be applied. Flame the bases and lids of the dishes with a lighter or gas burner using the tweezers to hold the bases and lids. Assemble while still hot.

3.6 Disposal of Contaminated Material

Note: To minimise the risk of infection from contaminated materials, take care not to touch contaminated membranes directly with your hands. Do not eat, drink or smoke while handling contaminated materials. Wash your hands immediately after you have touched any contaminated material and after you have finished your work.

Contaminated material, such as used membranes and pads, MUST be made safe before disposal. DO NOT discard non-sterile membranes and pads into the environment since they pose a major risk to public health.
After you have completed the analysis, stack the petri-dishes in the straps and sterilise the dishes and contents at 121°C for 30 minutes using an autoclave, steriliser or pressure cooker. Alternatively, plunge the petridishes, pads and membranes into boiling water and heat for at least 30 minutes (use a dedicated pan for this procedure. DO NOT use a pan that will subsequently be used for food preparation or other domestic purposes). After sterilisation, the used membranes and pads may be destroyed by incineration.
The petri-dishes must be carefully washed with detergent after use, rinsed with clean water and dried.

3.7 Absorbent Pads and Dispenser

The pads are supplied sterile in packs of 100 units. A pad dispenser is also supplied with the kit. Never leave the dispenser without a pack of pads attached as it will increase the possibility of contamination.
You might find it more convenient to dispense the pads into the petri dishes at your base to avoid the need to take the dispenser and pads into the field.

If it is necessary to dispense pads in the field, take care not to contaminate the dispenser assembly. If the dispenser is lost or damaged, pads may be dispensed in the field using the sterile tweezers (see Section 5.4.4[3] for sterilisation methods). Some kit operators prefer this method to using the dispenser.

3.8 Methanol Dispenser

The methanol dispenser is supplied with a plastic cap and dispensing nozzle. The dispenser should be half-filled with methanol using a small funnel, pipette or syringe to avoid spillage. Do not overfill the methanol dispenser as it may leak in hot weather.

To dispense methanol, lever the dispensing nozzle into the upright position with the tip of the tweezers. To seal off the flow of methanol, push the nozzle down into the recess in the cap. Be sure to close the dispensing nozzle after using the kit as the methanol will evaporate.

Note: Methanol is highly flammable. Keep methanol away from naked flames.