WEDC

MOBILE NOTE 51

Membrane filtration

Introduction

Faecal matter is an indicator of many diseases that are transmitted by the faecal-oral route, so if faecal organisms are found in water we can assume that disease-causing organisms, commonly called 'pathogens', are also present. Membrane filtration is a means of testing the quality of water for faecal contamination and therefore a way of determining whether a water supply is safe, or whether the water needs treating before consumption. This note presents an overview of the process.

Loughborough University

Contents

This mobile note is a transcript from the WEDC film of the same title which is available here: http://wedc.lu/membrane-filtration

How membrane filtration works

Water is filtered though a membrane having pore sizes smaller than the size of a bacterium. As water is filtered, bacteria are collected on the surface of the membrane. The bacteria can then be incubated to produce visible colonies suitable for analysis.

Thermotolerant faecal coliforms are indicator bacteria, which can form colonies at 44 degrees centigrade, and these are the colonies that provide evidence of faecal contamination of water.

The analysis of colonies of faecal indicator bacteria that survive an incubation period indicates that faecal matter is likely to be present in a water sample.

So this microbiological process is about producing samples of bacteria for analysis, not samples of clean water.

There are several stages to the process of sampling bacteria using a membrane filter:

- Preparation – assembling the kit and preparing the food
- Sterilization of the equipment
- Collecting the water sample
- Filtration
- Growing the bacteria
- Counting the colonies of bacteria

Preparation – assembling the kit and preparing the food

Here's how to go about it using a field testing kit which typically contains membrane lauryl sulphate broth (MLSB) which is 'food' for the bacteria and

can be prepared in the field (Figure 1). Alternatively other standard nutrient materials already prepared can be used.

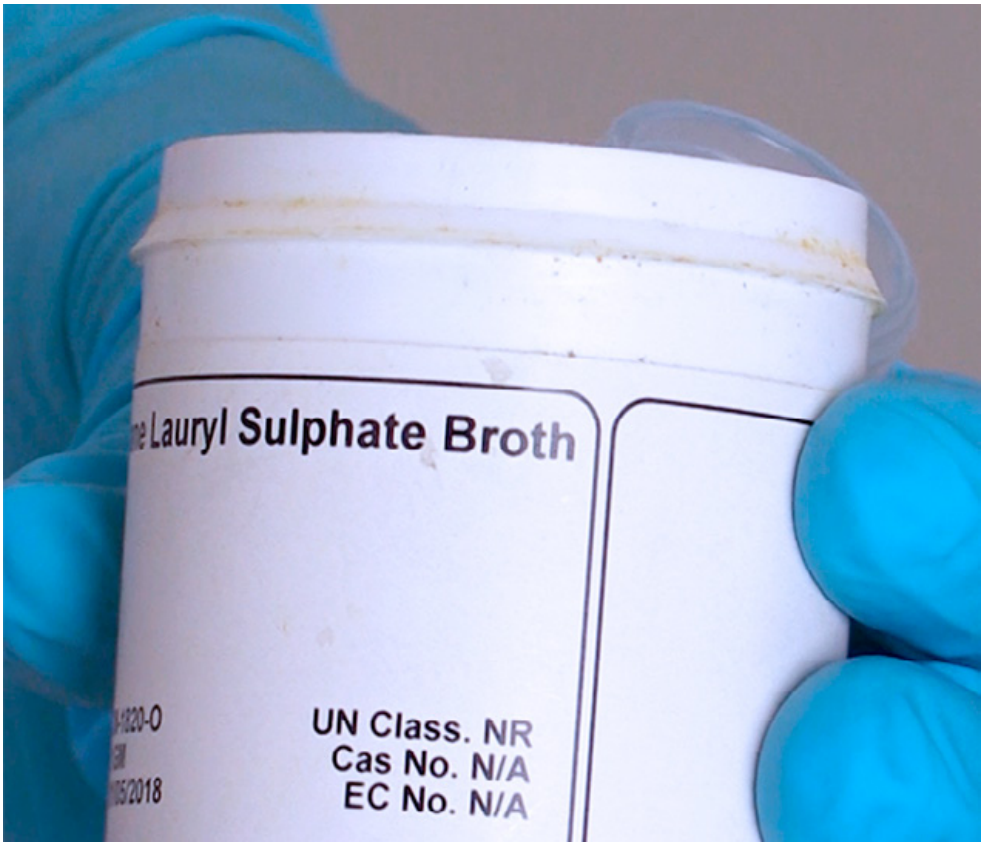

Figure 1. Membrane lauryl sulphate broth

Most field testing kits include:

- a media measuring device
- an absorbent pad dispenser;
- petri dishes;
- a membrane filtration unit;

- a hand vacuum pump;
- forceps;
- membrane filters;
- an incubator with mains power supply, battery and car charging lead;
- a magnifying hand lens;
- a marker pen;
- a lighter;
- a sterile syringe; and
- an instruction manual.

Figure 2. A field testing kit

The instruction manuals provide detailed instructions, so this film presents an overview of the process. Always refer to the manual provided by the manufacturer of the field testing kit you use.

To grow bacteria, you will need a supply of food. If you are to prepare your own, pour the membrane lauryl sulphate broth into a media measuring device followed by clean hot water. Use the amounts indicated in the instruction manual for your kit, as these can vary (Figures 3 and 4).

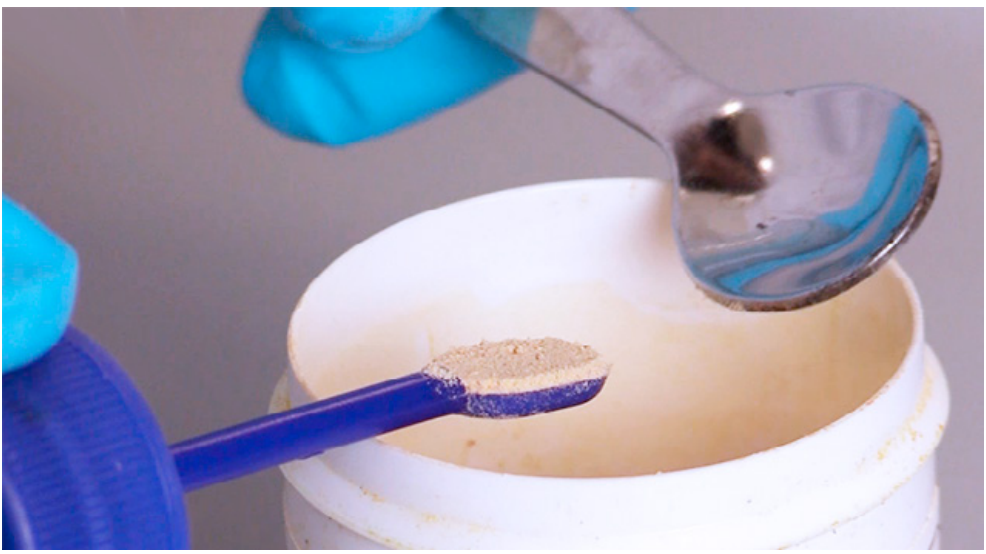

Figure 3. Measuring out the amount of MLSB

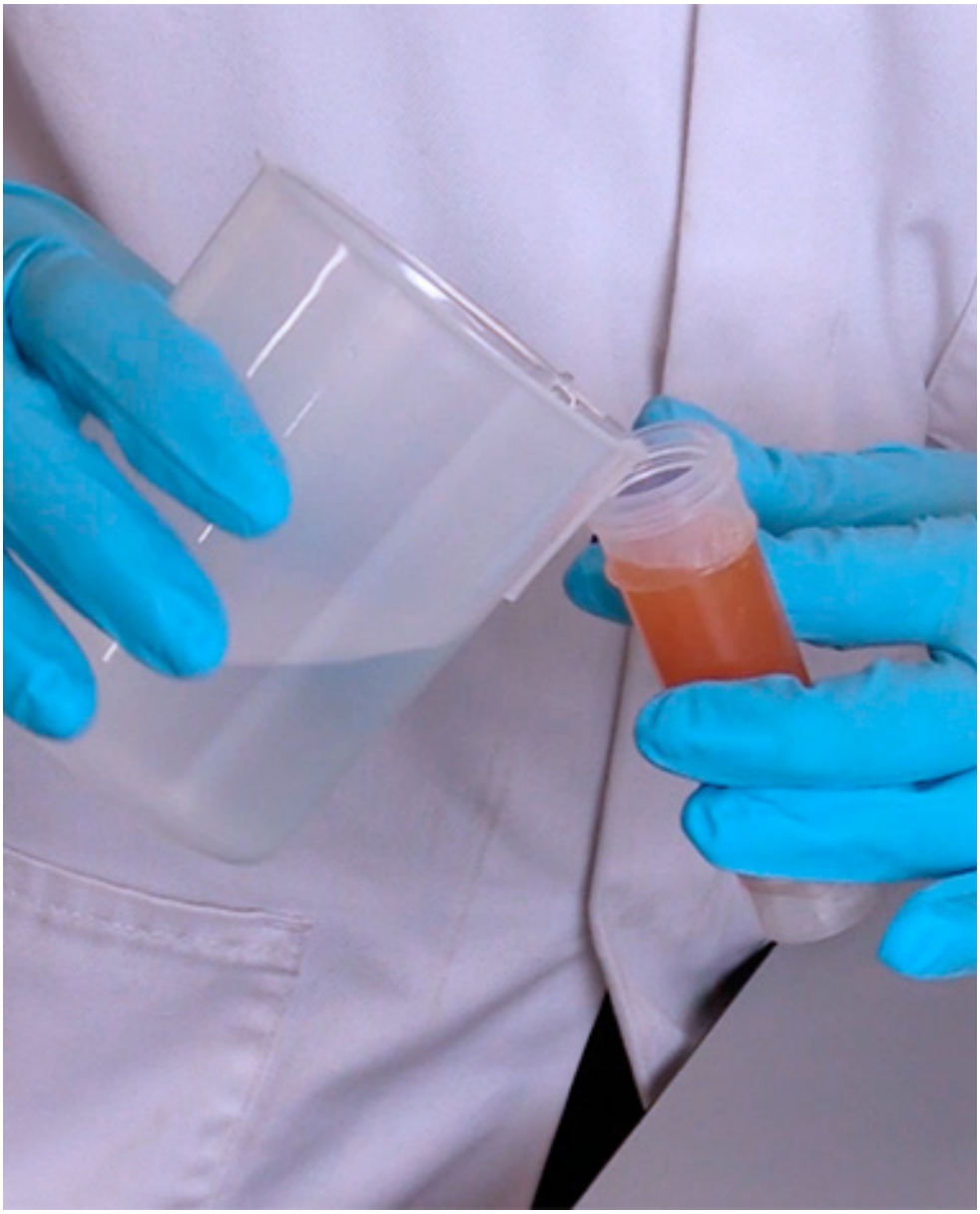

Figure 4. Pouring the MLSB into a media measuring device

Shake the media measuring device to ensure that all the powder is dissolved. As it does so, you will notice that the liquid turns red (Figure 5).

Figure 5. Shake the measuring device well

Sterilization of the equipment

All utensils used in the process need to be sterilized to prevent contamination from bacteria external to the process.

So sterilize the petri dishes and the filtration unit. Sterilize the filtration unit first then, as it cools, prepare the petri dishes.

To sterilize the filtration unit, squirt a small amount of methanol into the bottom reservoir and ignite it (Figure 6).

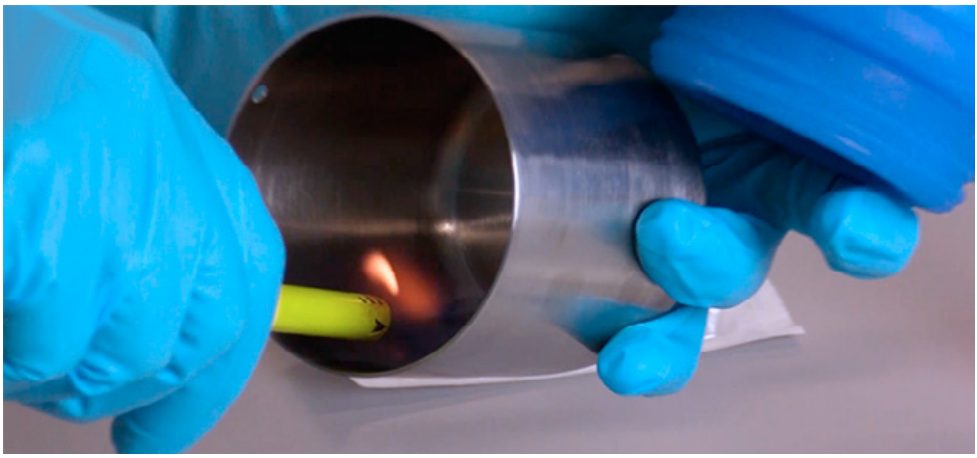

Figure 6. Sterilzing the filtration unit

When alight, turn the filter unit upside-down over the reservoir. Methanol is used because formaldehyde gas is released when burned without oxygen. Formaldehyde gas acts as a strong disinfectant to sterilise the top section of the filter.

In this demonstration, we are using aluminium petri dishes, which can be sterilized in the same way. At least two petri dishes should be prepared per sample so that you can cross-check the results. Once ignited and the methanol is burning well, place the lid over the bottom of a petri dish (Figures 7 and 8).

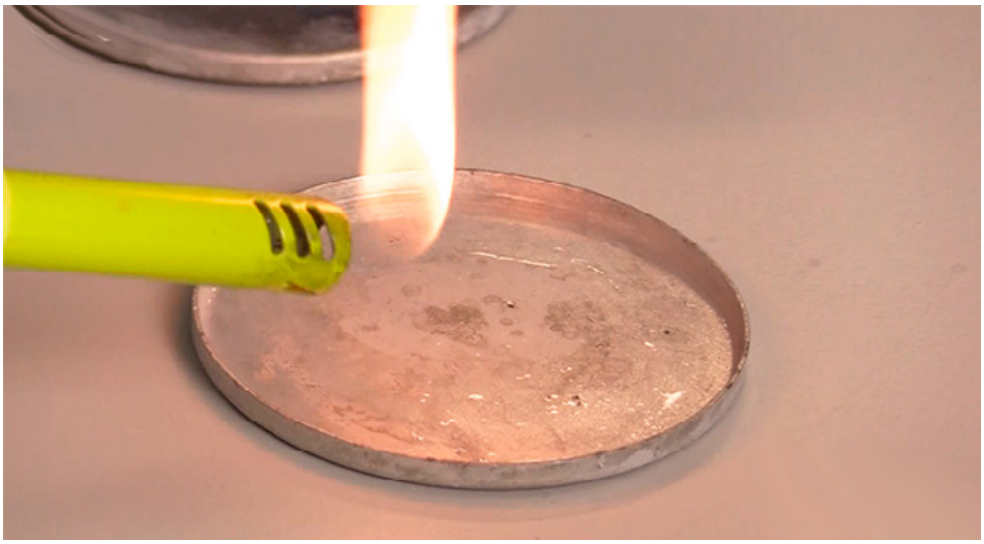

Figure 7. Sterilizing a petri dish

Note that it is not the heat that sterilizes the filter unit or the petri dish, although that does contribute to sterilization. It is the production of the formaldehyde gas that ensures the items are adequately sterilized.

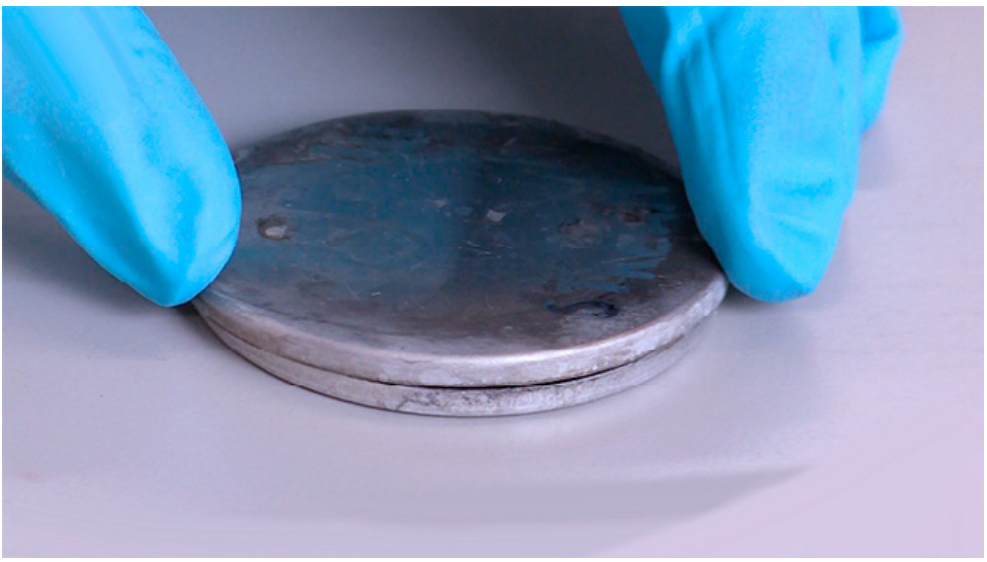

Figure 8. Placing the lid over the sterilized petri dish

As soon as a petri dish has cooled, uncover it and dispense an absorbent pad inside it, to absorb the membrane lauryl sulphate broth. The broth is the medium that encourages the bacteria to form colonies. Using a sterile syringe, dispense the broth onto the pad, covering it so that it is well soaked (Figure 9).
It is important to avoid touching the absorbent pad or the inside of the petri dish, as this could introduce other bacteria accidentally.

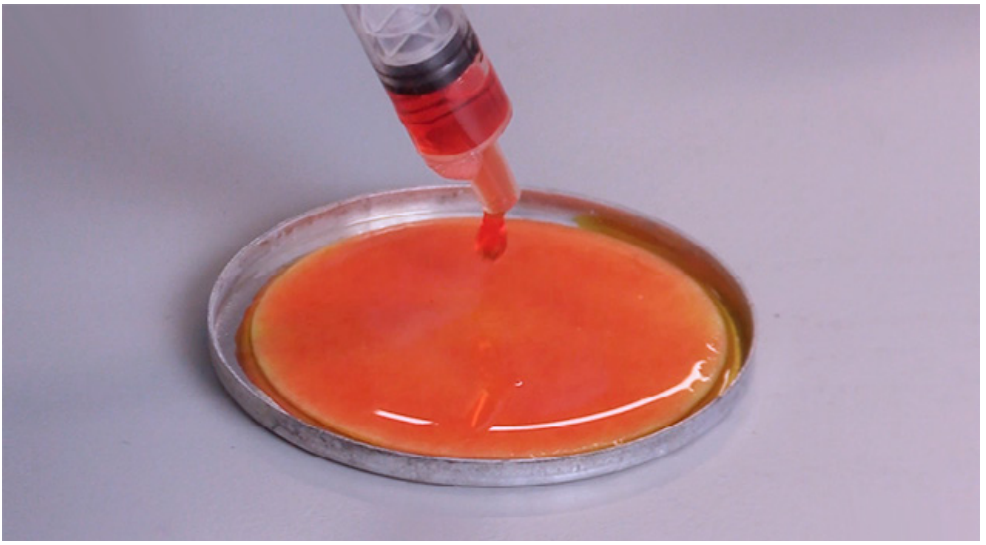

Figure 9. Dispensing the broth on an absorbent pad placed in the petri dish

Replace the lid so that bacteria from the atmosphere cannot be trapped inside the petri dish. It is not completely closed, however, to prevent adhesion of the medium to the lid (Figure 10).

Figure 10. Resting the lid over the bottom section of the petri dish

When the filtration unit has cooled down it will be ready to filter. Release the upper section of the unit filter so that it is just resting in place (Figure 11).

Sterilized forceps are needed to manoeuvre the absorbent pad into the

unit. If this is done manually, without sterile forceps, bacteria on your hand will contaminate the pad.

Figure 11. The upper section of the filtration unit resting in place.

Ethanol is used to sterilize the forceps. Leave the forceps in the ethanol until required.

Igniting the ethanol on the surface of the forceps sterilizes the forceps. This should be done every time they are used. Shaking the forceps will cool them down ready for use.

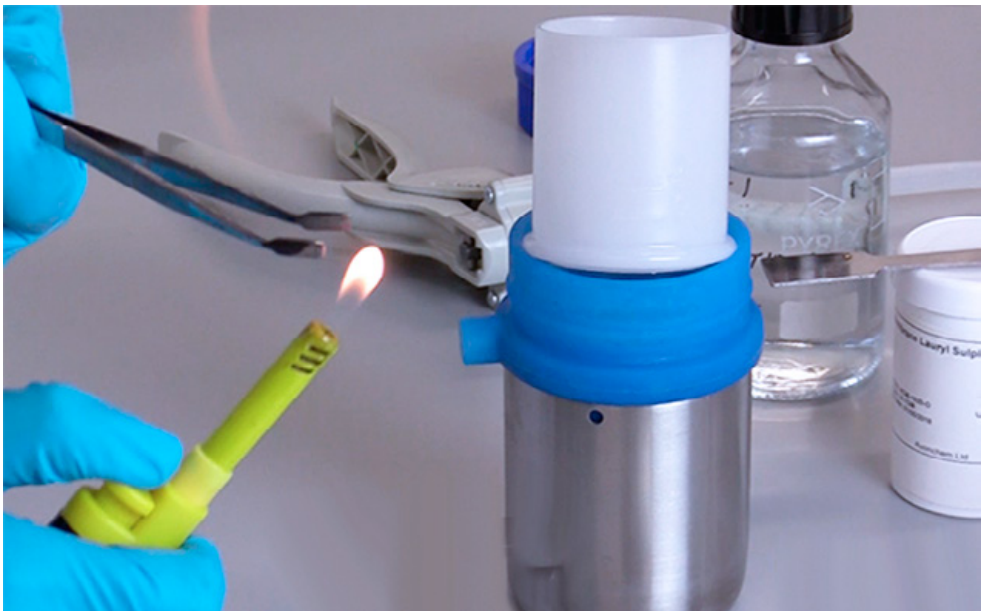

Figure 12. Sterilizing the forceps

Collecting the water sample

Refer to the separate film or guide about water sampling to find out how best to collect samples from a stream, a well, a handpump and water containers, which may also hold contaminated water. The film is availalbe at http://wedc.lu/water-sampling

Filtration

Now everything is prepared, and you have collected a sample of water, you can start the filtration process.

Place the membrane filter into the filter unit with the grid showing (Figures 13 and 14).

Filters come in sterilized sealed packs and each one has to be removed carefully so that other bacteria are not introduced into the filtering environment accidentally.

Figure 13. Placing the filter into the unit

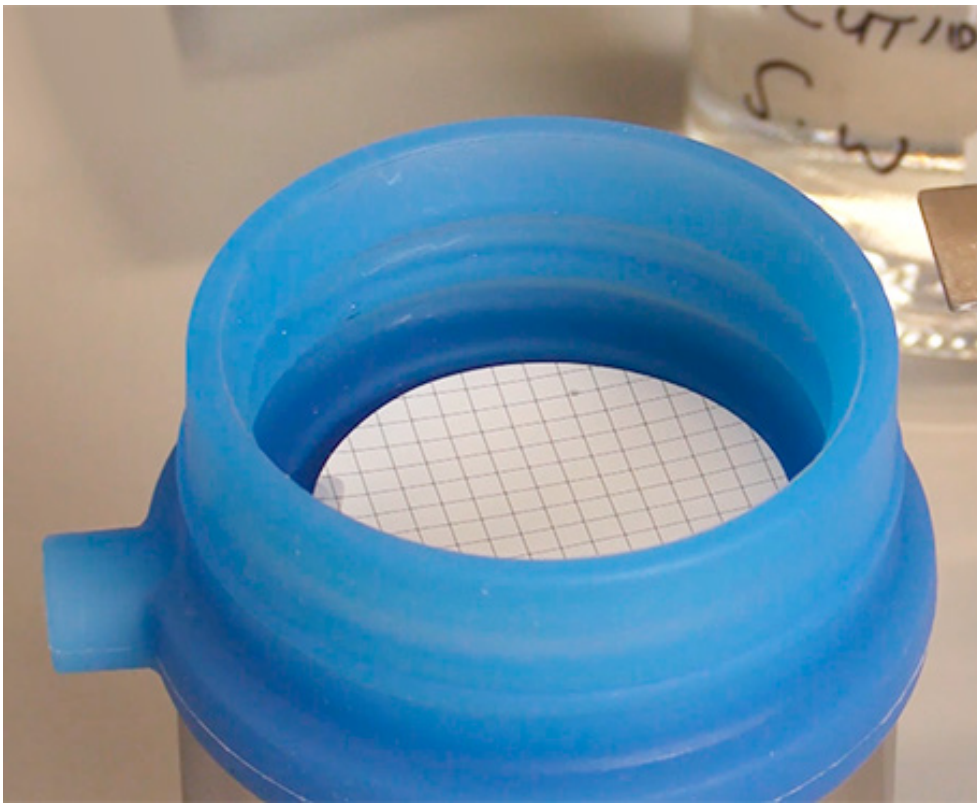

Figure 14. The filter placed in the unit

Shaking the water sample will make sure that the bacteria are evenly distributed, as they tend to settle at the bottom of standing water.

Next, pour the water sample carefully into the filtration until the 100 millilitre mark is reached, or to the depth recommended by the manufacturer of your test kit (Figures 15 and 16).

Figure 15. Pouring the water sample into the unit

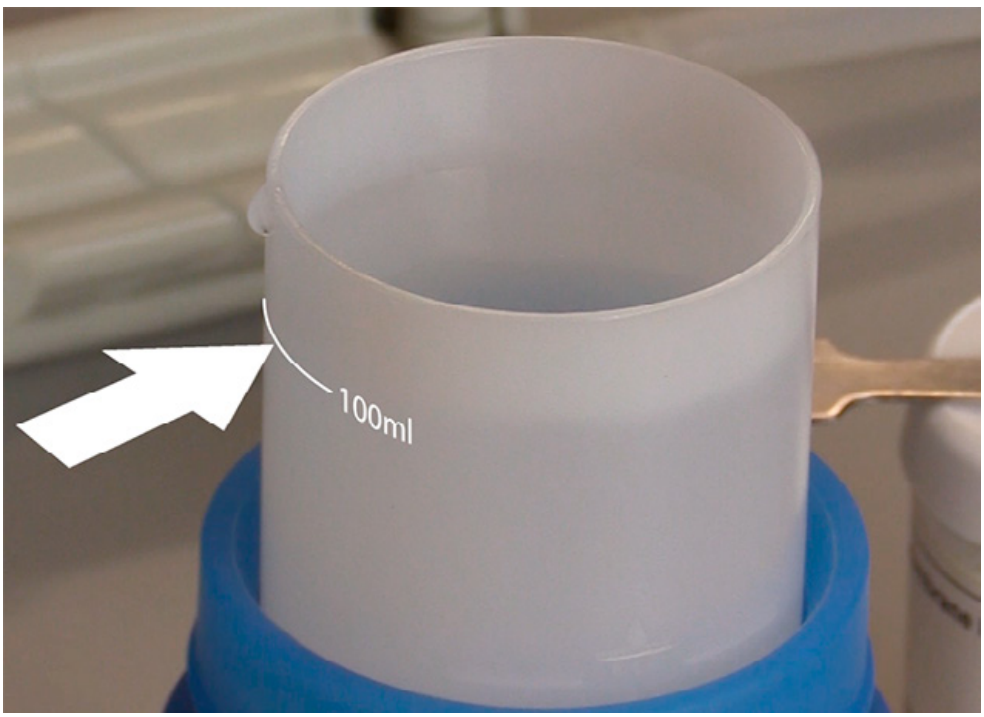

Figure 16. The 100ml level is reached

By attaching the hand pump, air is evacuated to produce a partial vacuum so that the water starts to filter into the base unit (Figure 17). When all the water appears to have been filtered, the unit should be shaken well to ensure that any residual water also passes through.

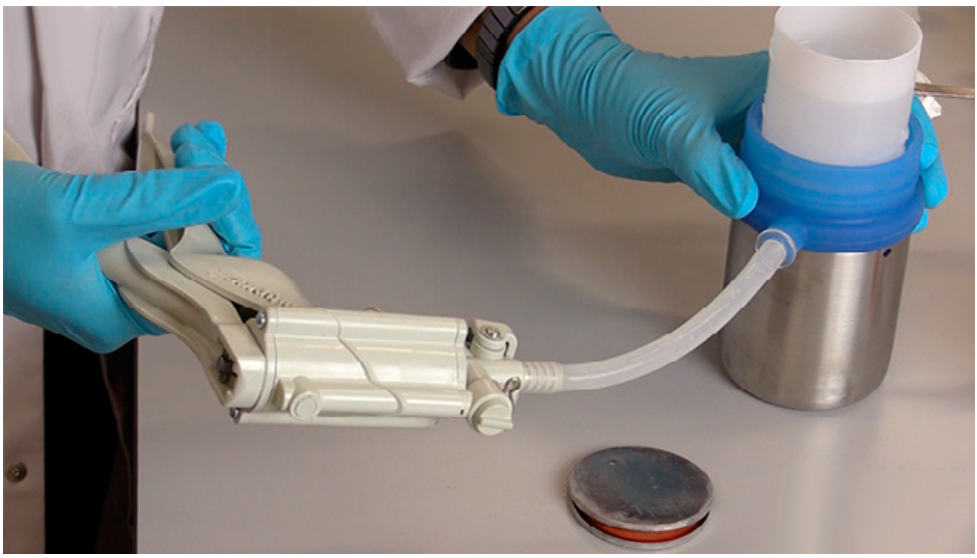

Figure 17. Operating the vacuum pump

As the water sample is poured through the filter, the bacteria are retained on the membrane, along with any suspended solids.

When you are sure that all the water has been filtered, release the top section of

the unit, sterilize the forceps again and shake them to cool them down.

Using the forceps, carefully remove the membrane filter, then roll it gently onto the absorbent pad in a sterilized petri dish and replace the petri dish lid. Rolling the membrane filter gently ensures that air bubbles do not get trapped underneath it (Figure 18).

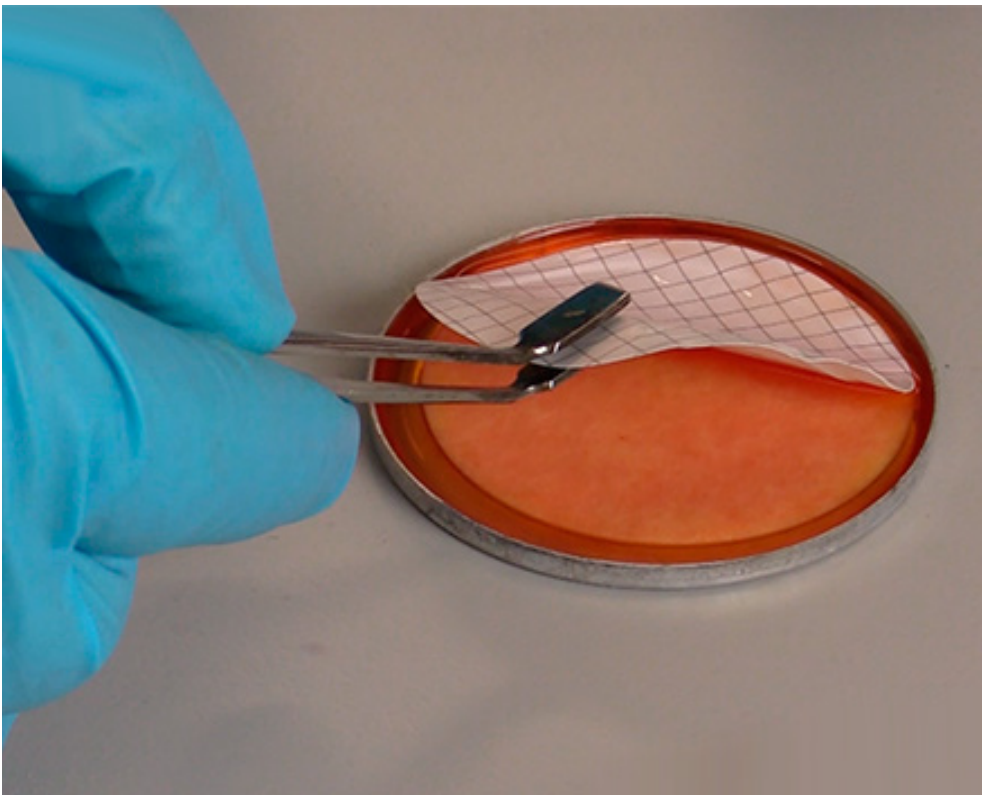

Figure 18. Laying the membrane carefully onto the absorbent pad

Growing the bacteria

Now label the petri dish and add your initials. Provide details to identify the source of water, and the date of collection. Then leave it at room temperature for up to 4 hours so that the bacteria have time to recover from the stress of the filtration process before incubation.

Figure 19. Leave the labelled petri dish for four hours prior to incubation

The incubation unit provided in most testing kits will hold up to 15 petri dishes so several samples can be tested at the same time.

Here, for demonstration, there is only one (Figure 20). Follow the instructions given in the incubation guide to operate the incubator (Figure 21).

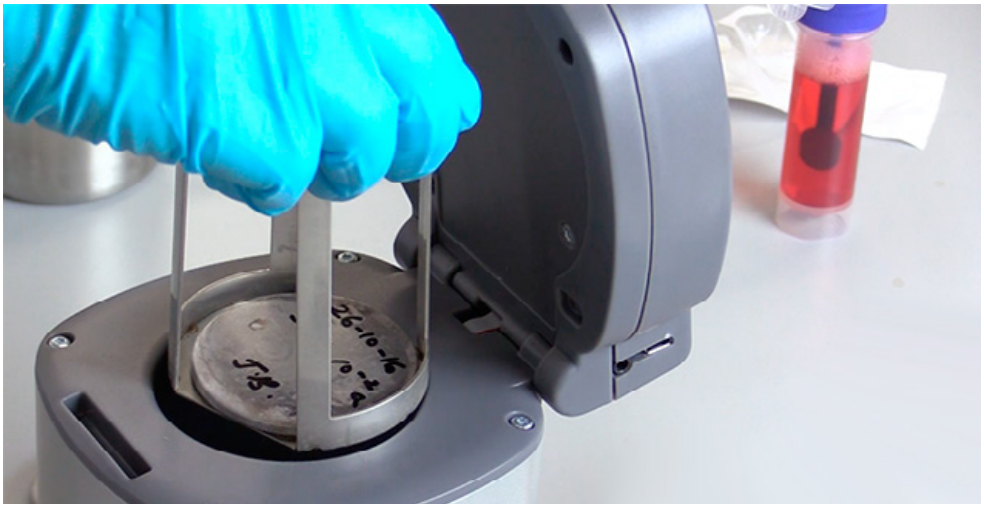

Figure 20. Placing the petri dish into the inubation unit

Figure 21. Operating the incubation unit

After 14 hours in the incubator at a temperature of 44 degrees centigrade the samples can be inspected. If thermotolerant coliforms are present they will have formed into yellow colonies. Pink colonies indicate that the water was contaminated by other coliforms.

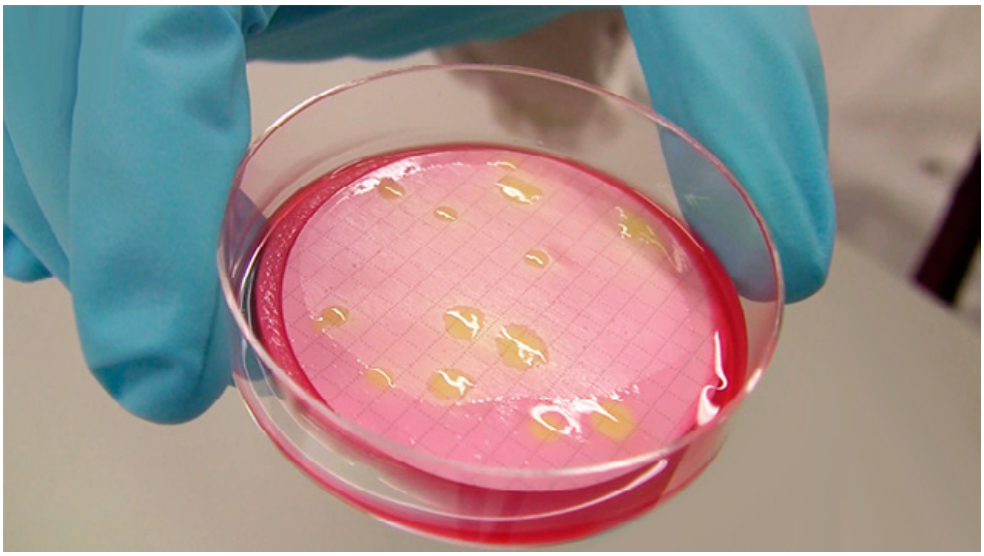

Figure 22. Colonies are yellow about the size of a drop of water

Counting the colonies of bacteria

It is usually possible to count the number of colonies of bacteria that have survived filtration and incubation, as each colony

resembles a droplet of water. If a petri dish is filled with colonies too many to count, the number of colonies should be recorded as T.N.T.C, meaning 'Too Numerous To Count'.

In this situation it is possible to dilute your sample using water that is free from bacteria, to obtain a more manageable number of colonies.

The water that was collected in the reservoir can be used to dilute water samples if required, as it has been filtered and is free from bacteria.

When dilution is necessary, typically 10 millilitres of a water sample could be mixed with 90 millilitres of water containing no bacteria to produce a 1 to 10 dilution, or 1 millilitre of a water sample could be mixed with 99 millilitres of water containing no bacteria to produce a 1 to one hundred dilution.

The numbers of bacterial colonies on the petri dishes for these dilutions should then be multiplied by 10 and one hundred respectively to give the number of bacteria present in an undiluted on hundred millilitre sample of water.

Calculations, taking into account any dilution, can then be made to give a rudimentary indication of the level of contamination. For detailed analysis the sample should be passed to a trained microbiologist.

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