Inter Aide Sierra Leone

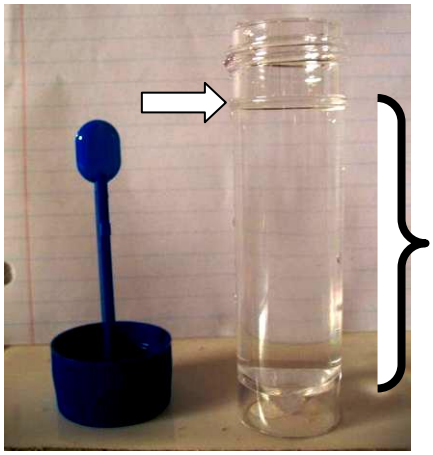
Laboratory Work Guideline
-
Water Quality Test

European Union

Day 1

I. Preparation of the culture medium

0) Wash and clean your hands and the worktop of the lab. Make some clear water (without particles) boil during at least 15 minutes to sterilize it. Then leave to cool. Clean the metal sample cup with alcohol and a tissue.



1) To make the culture medium for 16 petri dishes, you need 50 ml of sterilized water. To measure this volume you can help yourself with the plastic test tube. If you fill it up to the upper mark you will get 25 ml. So that, you need the content of 2 plastic test tubes. Pour this 50 ml in the metal cup.

2) Weigh the amount of Lauryl Sulphate powder you need. For 50 ml of culture medium, you need 4 g of Lauryl Sulphate. Help yourself with the blue plastic spoon of the plastic test tube. You need 8 spoons for 4 g.

3) If you want to verify the weigh, put those 8 spoons of Lauryl Sulphate powder on a piece of paper. Switch on the balance and weigh to verify the amount of Lauryl Sulphate. Pour the Lauryl Sulphate in the metal cup into the 50ml of water. Shake it gently to dissolve the powder.

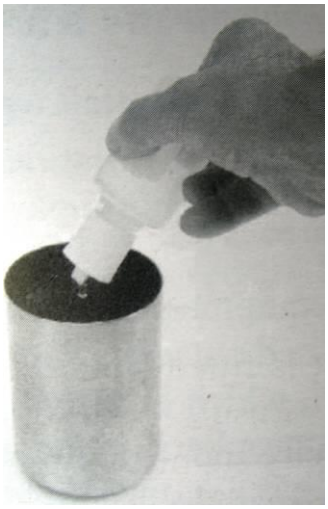
4) Pour some drops of methanol in the plastic bottle and shake it. Clean the cap with alcohol and a tissue. Let it dry.

Some pictures come from the Delagua Manual

5) Pour the culture medium solution into the plastic bottle and close the cap.

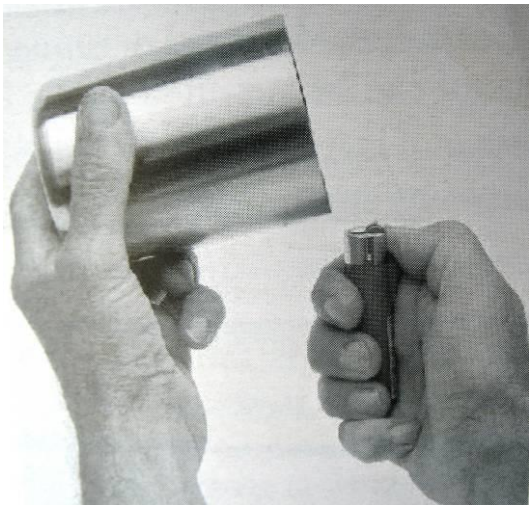
II. Sample processing

A) Sterilizing the Filtration Apparatus



0) Wash your hand and the environment

1) Clean with alcohol and a tissue the filtration assembly. Pour about 20 drops of METHANOL into the metal cup.



2) Carefully ignite the methanol in the sample cup. Allow the methanol to burn for several seconds.

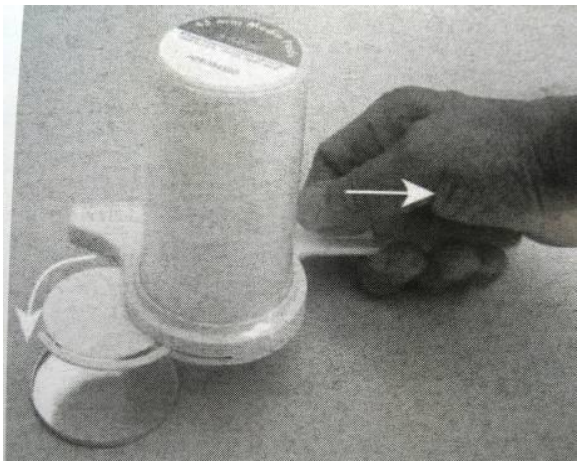
3) When almost completely burned up, place the filtration head over the cup and seal it. Use the plastic collar of the filtration assembly to secure it in the loose but not free position.

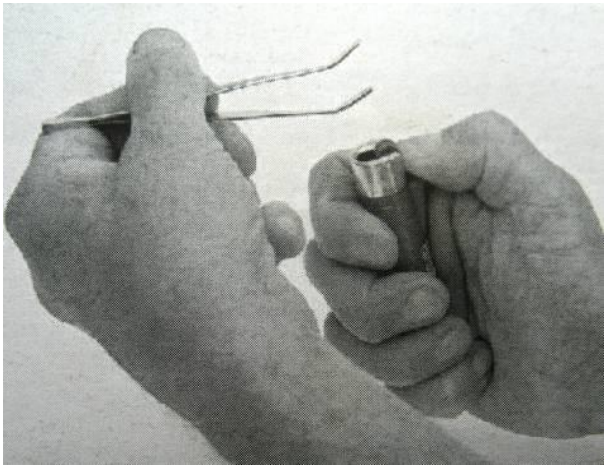
Keep the filtration sealed for at least 15 minutes before use

Do it after each sample

B) Petri dishes preparation

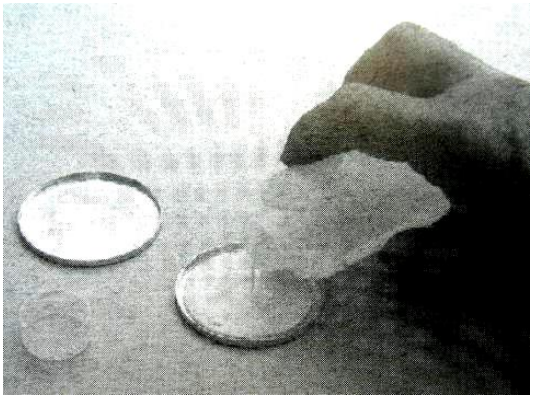
1) Lay the Petri-dishes on the work surface. Clean them with alcohol and a tissue.
Dispense one pad into every first Petri dish using the dispenser. **Do not touch the pad with your fingers.**





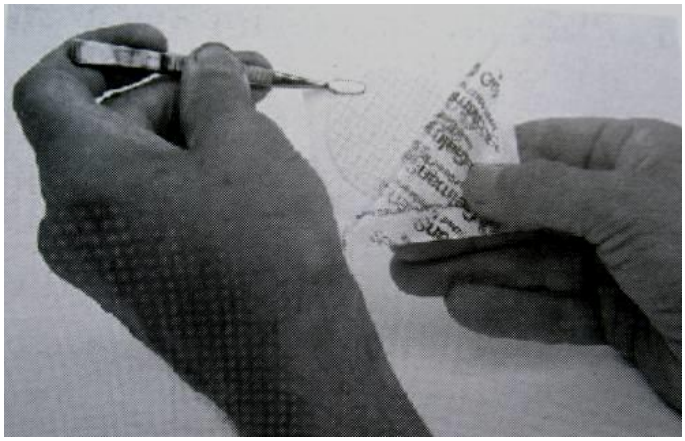
3) Flame the tips of the tweezers with lighter for 5 seconds and leave to cool. Keep it away from any contacts on any surface, hands or tool….

2) Pour the medium onto the absorbent pads, Leave a slight excess so that the pad will not dry during incubation. Cover them.



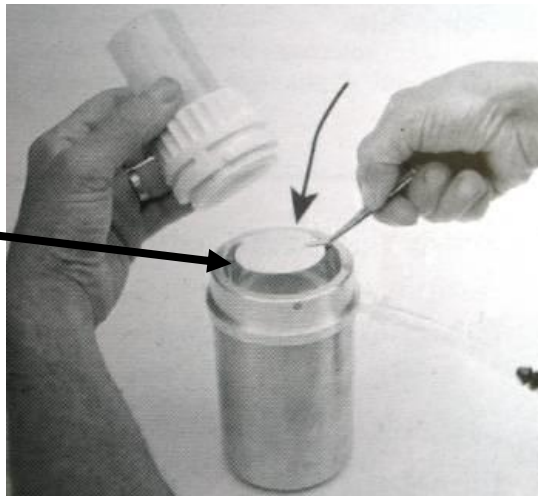
C) Filtration

1) Assemble the filtration apparatus. Unscrew the plastic collar and filtration funnel. Do not place these on any surface other than the filtration base.

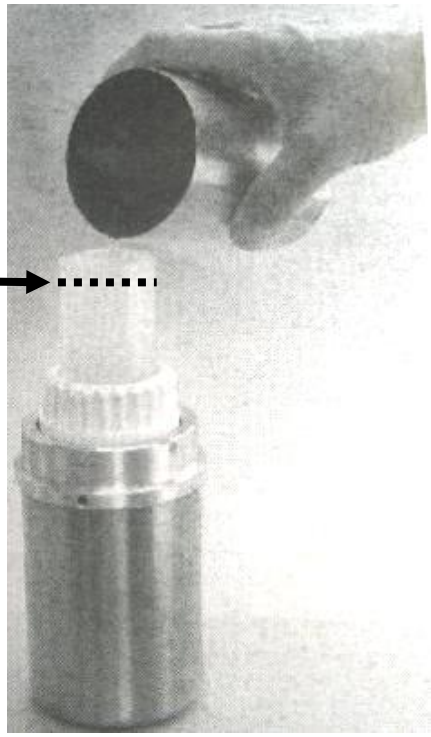


2) Use the sterile tweezers to remove a sterile membrane filter from the packet. Do not touch the membrane with your fingers.

3) Place the membrane onto the filter support.



4) Screw the collar tightly

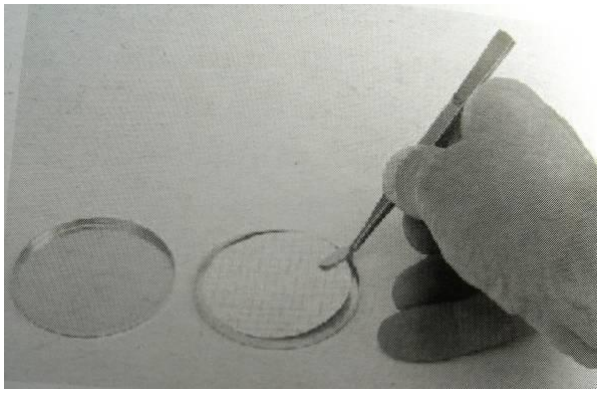


5) Pour the sample into the filtration funnel to the mark engraved on the funnel.
Take care not to allow debris to enter the funnel.

If you estimated that the contamination is potentially high, reduce the amount of water (10 ml for traditional water points or highly contaminated water points)
If the sample is potentially few contaminated (protected well) you can filter 100ml but try to have a sample of 50ml (it will reduce the number of colony and facilitate the

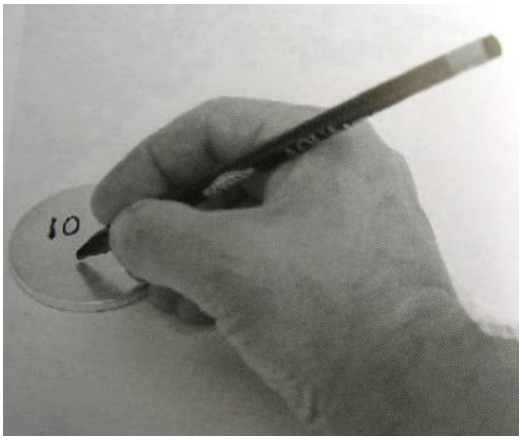
6) Connect the vacuum pump with the filtration base. Squeeze the pump to draw all the water through the filter. Stop pumping once the water has gone.

7) Unscrew the collar, remove the funnel and lift the membrane with the sterilized tweezers. Do not touch the membrane with your fingers



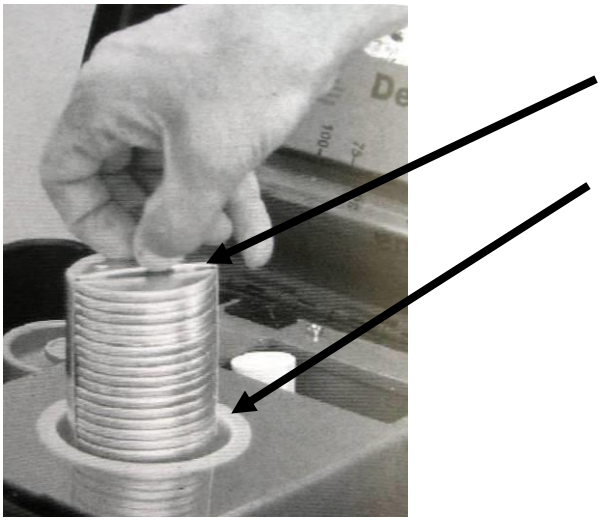
8) Lower the membrane on to and adsorbent pad in a Petri dish.

9) Replace the lid and mark it with the number of the sample.



10) Fill the Laboratory sheet with the number of the sample and the name of the water point of the water sample.

11) Before filtering a new water sample, sterilize the filtration apparatus once again. Once you have done every sample, do not forget to fill the laboratory sheet to record the information of the Petri dish samples.



Once you have finished, stack the Petri dishes. Place them with the lid uppermost into the carrier.

Return the carrier to the incubator pot.

Sterilise the filtration apparatus.

Wait for 60 minutes before switching on the incubator.

Incubate the samples for 16 to 18 hours at **44°C**.

Fill the Laboratory Sheet

D) Cleaning

Clean and disinfect with alcohol all glasses and caps used for taking water samples. Store them upside down on a tissue. Clean and disinfect the working surface and all tools you have used. Clean your hands with soap. Clean the door handle of the lab if you went out of the room during the experiment.

Day 2

III. Counting colonies

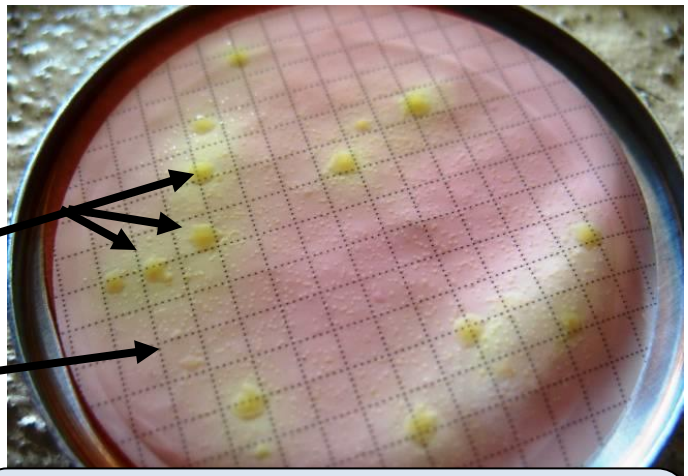
A) Observation

Do the counting as soon as possible after the Petri dishes have been removed from the incubator, as the **colours are liable to change** on cooling and standing.

Remove the Petri dishes from the incubator, remove the lid and observe the surface**. Count only yellow colonies > 1 mm diameter**.

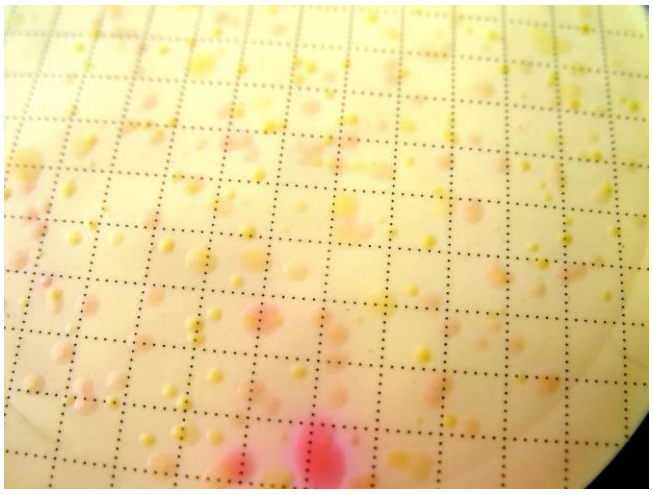
Do not count colonies that are transparent, red or any other colour – these bacteria do not ferment lactose and are not Thermotolerant Coliforms.

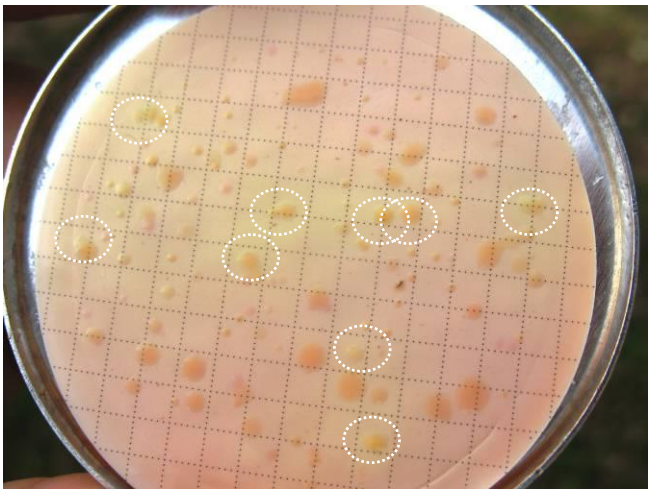
Fill the Laboratory Sheet with the colonies counting. Gridded membrane filters permit easier counting, count column by column.



Fecal coliforms *(Brit.: faecal coliforms) are bacteria part of total coliforms and are the only members of this group which can be found in human or animal feces. Therefore they can be used as an indicator of fecal contamination of the water and of possible pathogens. These coliforms are able to grow at* ***44°C*** *while it is not possible for other non-fecal strains. The main fecal coliform bacteria are Escherichia Coli (E. Coli).*

If the number of colony is to high, it is hard to distinguish the "yellow" thermotolerant coliforms form all other "clear" or 'red/orange" colonies. To facilitate the counting filtrate 50 ml of sample instead of 100ml





Left: picture of half a Petri Dish of a 100ml filtration culture. It is very hard to be precise on the number of "yellow" E.coli among the other "clear", pink and orange colonies

Right: picture of a Petri Dish culture of the same sample but for only 50 ml. It is possible to distinguish the different type of bacteria species and to identify thermotolerant coliforms

B) Disposal

Throw the contaminated materials (pads and filters) directly into the combustion drum outside. Do not use the common garbage bins. Sterilize every Petri dish by cleaning them entirely with a tissue and alcohol. Clean the inside of the incubator and the plastic cap. Clean and disinfect the working surface and all tools you have used. Clean your hands with alcohol. Clean the door handle of the lab if you went out of the room during the experiment.

C) Cleaning

Clean and disinfect with alcohol all glasses and caps used for taking water samples. Store them upside down on a tissue. Clean and disinfect the working surface and all tools you have used. Clean your hands with alcohol. Clean the door handle of the lab if you went out of the room during the experiment

D) Data Collection

Verify that Field Sheet and Laboratory Sheet are properly filled. Complete the Water Point Sheet with the results of the day. Create a new sheet if the village has never been tested before.

Laboratory maintenance

You should use a notebook especially to record any information on the experiments (date, time, information on mixing and solutions, samples and results). This notebook and pen and pencil you use should stay in the lab and not be used for other purpose. This will avoid any cross contamination.

Store the alcohol in a secure and cool place, a cupboard for example. Have a fire extinguisher in the lab and be trained to use it

Do not smoke, eat or drink in the lab

Keep the lab clean. Disinfect the working surface with alcohol before and after you work in the lab

Before any experiment, clean your hand with soap.

At the end of any experiment with contaminated materials, sterilize and disinfect and tools or surface which have been in contact with contaminated material (including the door handle). Wash your hand with soap.