

18. Water testing

The objective of bacteriological testing of water is to detect and determine the concentration of faecal bacteria in water supplies in order to:

a) check that the supply is free from pathogenic (disease-causing) organisms and therefore safe to drink,

b) assess faecal pollution of supplies.

The number of organisms excreted into the environment each day by every living creature is enormous.

At present it is not possible to identify pathogens in drinking-water quickly. The pathogens may only be present occasionally in the water although pollution by faecal matter may be occurring all the time.

Normal bacteriological procedures to test for pathogens in the water determine if faecal pollution has occurred. If faecal bacteria are present, then pathogens may be there too. (See Diagram A.)

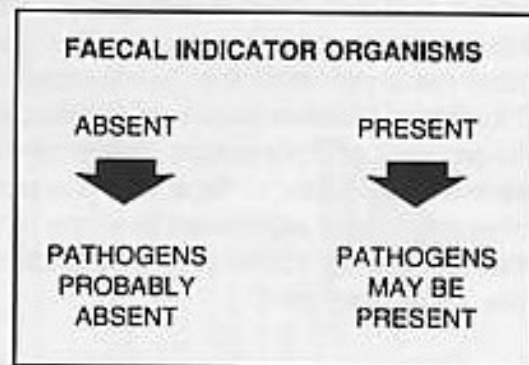


Diagram A

The most useful indicator organism for surveillance of bacteriological water quality are members of the faecal coliform group (See Diagram B).

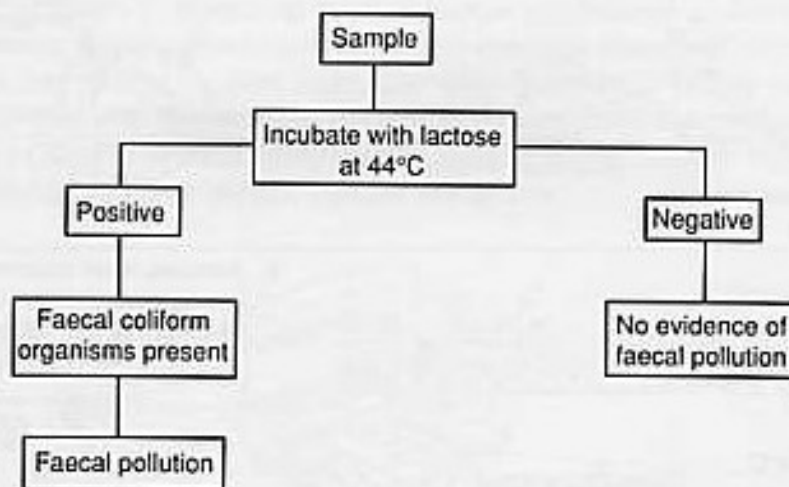


Diagram B

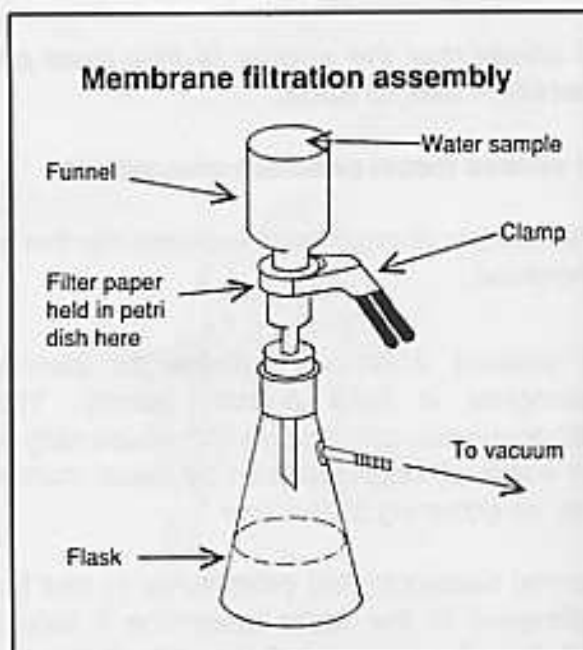
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There are two main methods used to detect and measure indicator bacteria in water:

- a) *The membrane filtration method.*
- b) *The most probable number (MPN) multiple-tube method.*

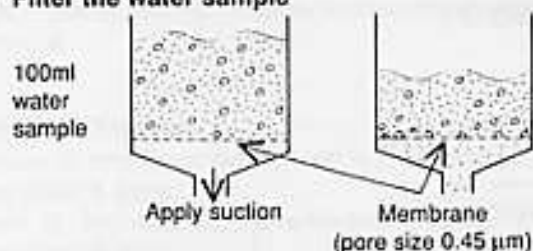
a) Membrane filtration

The most useful method for testing faecal indicator bacteria in drinking-water is by membrane filtration. This procedure involves filtering a measured volume of sample (100ml), or an appropriate dilution of it, through a membrane filter which has a pore size of 0.45 μm . Micro-organisms are retained on the surface of the filter which is then placed on an absorbent pad soaked in a suitable selective growth medium (containing lactose) in a glass, plastic or metal petri dish. It is then incubated at 44°C for faecal coliform detection. Any bacteria able to grow will multiply to form visible colonies on the membrane filter surface. The number of colonies counted is expressed in terms of the number present per 100ml of original undiluted sample (See Diagram C).



The determination of microbiological water quality by membrane filtration

1. Filter the water sample



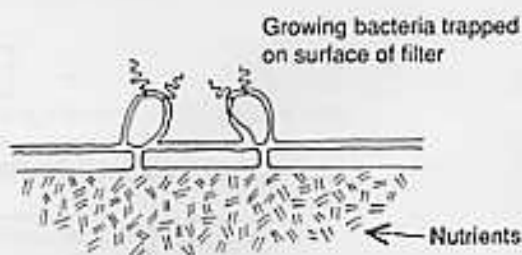
2. Supply the bacteria with nutrients



3. Incubate at 44°C in an incubator

(See Diagram F)

4. They grow and individual colonies can be counted after 24 hours



5. Assume each colony arose from a single bacterium. Calculate number of colonies per 100ml.

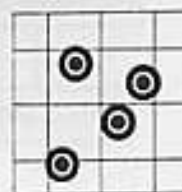


Diagram C

b) Most probable number (MPN) multiple-tube method

The multiple-tube method involves adding measured volumes of sample to sets of sterile tubes or bottles containing suitable liquid culture medium. Faecal coliform organisms produce acid and gas when incubated at 44°C. The gas production is detected by its appearance in a small 30mm inverted glass test tube (Durham tube) inserted into the tube or bottle before sterilisation (See Diagram D).

Acid is detected using various pH indicators. The number of tubes showing positive reactions is recorded at the end of the incubation period. An estimate of the most probable number (MPN) of organisms present in the original sample is obtained from statistical tables. A range of different dilutions should be used to ensure that both positive and negative reactions are obtained.

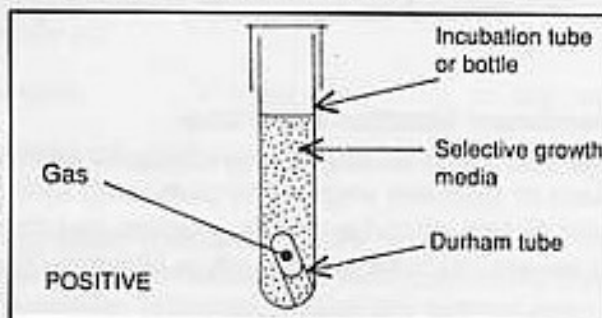


Diagram D

The multiple-tube fermentation or MPN technique is applicable to waters of all types and especially those with high turbidity. The equipment required is relatively cheap and simple, positive reactions are easy to read (See Diagram E). (One of the simplest procedures using 5 x 10ml of sample is described in detail by Mara in Cairncross and Feachem, 1978.)

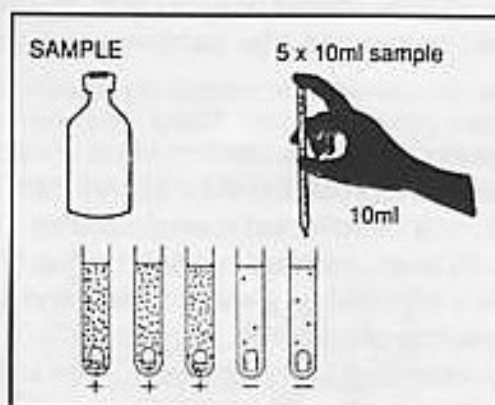


Diagram E

Incubators

The most difficult problem in operating bacteriological procedures in developing countries occurs with incubators. Battery-powered incubators have been developed in the past few years particularly for the membrane filtration technique (See Diagram F). They use an aluminium heating block, insulated and temperature controlled by electronic thermistors. The cheapest cost around £600 in Britain. Work is being carried out to provide cheaper heating systems for MPN and other procedures. For details, contact the author.

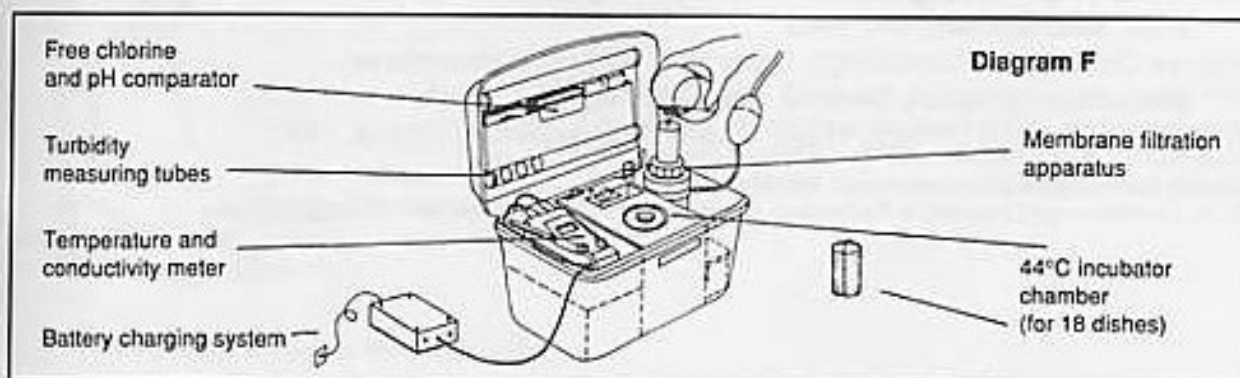


Diagram F

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Autoclaves

Autoclaves are another essential part of a bacteriologist's equipment. In the absence of a commercial autoclave, ordinary domestic pressure cookers are suitable for sterilising sample bottles, screw-capped bottles, measuring cylinders, pipettes, petri dishes, tweezers, dilution and rinsing fluids and membrane filtration apparatus prior to use. They should also be used to destroy bacteria developed after the testing procedures have been carried out.

Membrane filtration apparatus

There are several systems available for filtering. These are made from polycarbonate plastic, glass or stainless steel. They come with syringes, pre-packed sterile selective growth media, petri dishes, absorbent pads, pipettes and membrane filters produced specifically for field use by several manufacturers such as Millipore, Sartorius, Oxoid, Nucleopore, Gelman and others.

General hygiene and cleanliness

The work area should be kept clean and free from dust and draughts. Hands should always be washed before and after sampling and analysing.

Samples

The sampling procedures are as important as the analysis. Samples for bacteriological analysis should only be collected in sterile bottles. Do not waste time and effort in testing samples which have not been collected in sterile bottles or improperly handled. Samples should be taken from a variety of points on a water distribution system. The reader is referred to more detailed texts on sampling procedures.

Water sampling is considered further in Technical Brief No.20.

References and further reading

- APHA/AWWA/WPCF, *Standard Methods for the Examination of Water and Wastewater*, 16th ed, Washington, DC, APHA, 1990.
- Mara, D.D. in Cairncross, S. and Feachem, R., *Small Water Supplies*, Ross Institute, London, 1978.
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