Method 9.2 – Drinking water and effluent: bacteria by the membrane filter method

1. Rationale

The method is used for the determination of the total coliform bacteria, faecal coliform bacteria and the standard plate count for bacteria in water.

2. Principle

Samples are filtered through sterile membranes of known pore size. The organisms are retained on the membrane surface and are subsequently detected as colony forming units growing on a nutrient pad which is specific for the test.

Sterile glassware and an aseptic technique must be used throughout the method.

3. Identification

3.1 Colony count

A colony count is the number of colony forming units (CFU) on the membrane. The results are reported as CFU per 10 g of sample.

3.2 Total coliform bacteria

E. coli and coliform bacteria develop sharply contoured, pink to dark red colonies with a golden-green metallic sheen (Fuchsin sheen) and a dark red point on the underside of the membrane filter which is situated on the appropriate nutrient pad. The sheen area may vary in size from a small pinhead to complete coverage of the colony surface. Colonies which lack sheen are considered to be non-coliform.

3.3 Faecal coliform bacteria

E. coli and faecal coliforms develop colonies that exhibit shades of blue or have a blue centre with a white periphery with diameters of 1 - 2 mm when grown on a membrane filter placed on the appropriate nutrient pad; non-faecal coliform colonies are grey to cream coloured and are not evaluated.

3.4 Standard plate count

The colonies of bacteria are stained red by TTC reduction.

3.5 Membrane filter

Membrane filters are thin porous discs (about 150 µm thick) composed of cellulose acetate or other cellulose esters or polycarbon. They are sterile and of known pore size. Bacterial cells are retained on the membrane surface where they may be cultivated or examined *in situ*. These retained cells lie over capillaries that are perpendicular to the filter surface. When the membrane is placed in contact with a suitable culture medium, nutrients are provided to the cells *via* the capillaries. Each individual cell will then, after incubation at the appropriate temperature, produce a single colony. The retention of cells

on the membrane filter is quantitative, and colony counts of bacteria are directly related to the volume of sample filtered. Membrane filters are not reusable.

4. Apparatus

4.1 **Apparatus for dry sterilisation** at 170 ± 5°C for glassware

4.2 **Autoclave** for sterilisation at 121 ± 2°C for water

4.3 **Incubators**: operated at 37 ± 1°C, 44.00 ± 0.25°C and 30 ± 1°C

4.4 **Graduated pipettes**: 1, 10 cm^3

Protect the mouth end of the pipettes with cotton wool plugs.

4.5 **Colony counting equipment**

4.6 **Test tubes**

4.7 **Filter funnel**, base with support and clamp

The filtration unit can be stainless steel, autoclavable plastic, glass or sterile disposable plastic, 50 mm ϕ with a capacity of 100 cm^3. Assemble loosely and protect openings with tin foil prior to sterilization.

4.8 **Flask** for mounting filter apparatus: 200 cm^3 with side arm

4.9 **Vacuum line**

4.10 **Forceps** for handling membranes

Sterilize by dipping the ends into ethanol and then igniting the adherent liquid.

4.11 **Bunsen burner**

4.12 **Sample bottles**: 200 cm^3

Use glass sample bottles, containing sodium thiosulphate solution to neutralise chlorine. Cover the closure and neck of the bottle with tin foil prior to sterilization.

4.13 **Heat sensitive tape** that changes colour to indicate a successful sterilisation cycle

4.14 **Volumetric flask**: 100 cm^3

5. Reagents

5.1 **Sterile distilled water** autoclaved at 121 ± 2°C for 15 minutes

5.2 **Disinfectant**

Commercially available household disinfectant such as “Jik”.

5.3 Culture media

Use commercially available nutrient pad sets impregnated with dehydrated nutrients in Petri dishes with membrane filters, 0.45 µm pore size, 45 mm ϕ (preferably marked with a grid).

The following nutrient pad sets are available from Sartorius:

Endo SM 140 53 ACN for total coliform bacteria
M-FC SM 140 68 ACN for faecal coliform bacteria
Standard TTC SM 140 55 ACN for standard plate count

Flood the used plastic disposable petri dishes with disinfectant prior to disposal.

5.4 Alcohol

Alcohol (ethanol, CH_3CH_2OH) is a flammable solvent and toxic and should be handled with care.

5.5 Sodium thiosulphate (100 g/litre)

Sodium thiosulphate ($Na_2O_3S_2$) is a mild irritant and may cause vomiting when swallowed.

Weigh 10 g of sodium thiosulphate and transfer to a 100 cm^3 volumetric flask. Make to the mark with distilled water.

6. Procedure

6.1 Sample collection

Collect representative samples in sterile 200 cm^3 sample bottles containing sodium thiosulphate to neutralise the chlorine present in the sample. Use twice the quantity of sodium thiosulphate necessary to react with the expected total amount of chlorine in the sample. For example, the presence of 0.8 cm^3 of a 100 g/litre solution of sodium thiosulphate is sufficient to neutralise up to 60 mg/litre of residual chlorine in 1 litre of water without affecting the microbiological quality.

6.2 Test conditions

Start the microbiological examination within 6 hours (24 hours if samples are kept between 1 and 10°C, but not frozen) of the time of sampling. The temperature of the samples should preferably be kept below 10°C during transportation and storage.

6.3 Preparation of the workplace

Clean and disinfect the working area before commencing analysis. Clearly mark the sterile Petri dishes with the sample name.

6.4 Medium preparation

Wet the nutrient pads with 3.0 - 3.5 cm^3 of sterile distilled water. Ideally a slight excess of liquid should be visible at the edge of the pad.

6.5 Glassware preparation

All glassware should be dry sterilised (170 ± 5°C) for 2 hours.

6.6 Sample preparation

Add 0.5 cm^3 of the water sample to each of two sterile test tubes respectively containing 9.5 cm^3 sterile distilled water.

If an effluent sample is being analysed, a dilution series needs to be prepared. Add 9 cm^3 sterile distilled water to each of five sterile test tubes. Add 1 cm^3 of the water sample to one test tube and mix well. Remove 1 cm^3 of the mixture and add it to the next tube and continue in this way. Remove 1 cm^3 of the mixture from the fifth dilution and discard. Each test tube should therefore contain 9 cm^3 of liquid.

Include one sterility check in every ten samples by using sterile distilled water instead of sample.

6.7 Filtration and incubation

Connect the filter apparatus to the vacuum line. Place the membrane centrally on the base with sterile forceps. Place the sterile funnel on the top and clamp in place.

Invert the bottle containing the sample about 25 times to distribute any deposit uniformly throughout the sample. Pour the sample into the funnel, apply the vacuum and filter the sample. Rinse the funnel with sterile water. Turn off the vacuum. Remove the funnel and use flamed forceps to place the membrane, grid side up, on a moistened nutrient pad. When positioning the membrane on the culture plate ensure that no air bubbles are trapped between them.

6.7.1 Total coliform bacteria

Filter 100 cm^3 of the undiluted water sample using Endo nutrient pad sets. Incubate at 37 ± 1°C for 18 to 24 hours.

6.7.2 Faecal coliform bacteria

Filter 100 cm^3 of the water sample using M-FC nutrient pad sets.

If an effluent sample is being analysed, filter each of the dilution series through a membrane filter starting with the most dilute. Also filter 10 cm^3 and 100 cm^3 of the undiluted sample through another two membrane filters.

Incubate at 44.00 ± 0.25°C for 22 to 24 hours.

6.7.3 Standard plate count

Dilute two lots of 0.5 cm^3 of the sample with 9.5 cm^3 sterile distilled water. Filter the diluted samples through two separate membranes. Incubate at 30 ± 1°C for 48 hours.

Flood the used plastic disposable petri dishes with disinfectant prior to disposal.

7. Calculations

7.1 Dilution factor for effluent samples

1 cm^3 sample diluted to 10 cm^3	dilution factor = 1×10
1 cm^3 of [1×10] diluted to 10 cm^3	dilution factor = 1×10^{-1}
1 cm^3 of [1×10^{-1}] diluted to 10 cm^3	dilution factor = 1×10^{-2}
1 cm^3 of [1×10^{-2}] diluted to 10 cm^3	dilution factor = 1×10^{-3}
1 cm^3 of [1×10^{-3}] diluted to 10 cm^3	dilution factor = 1×10^{-4}

8. Expression of Results

8.1 Total coliform bacteria

Count the colonies on the membrane using the colony counting equipment. The total coliform bacteria shall not exceed 5 per 100 cm^3 of water.

8.2 Faecal coliform bacteria

Count the colonies on the membrane using the colony counting equipment. If an effluent sample has been analyzed, multiply the result by the dilution factor (7.1).

Faecal coliform bacteria shall not be present in 100 cm^3 of water.

8.3 Standard plate count

Count all the colonies on both the membranes using the colony counting equipment. It is recommended, but not essential, that the standard plate count should not exceed 100 per 1 cm^3 of water.

If the test sample taken complies with the requirements, the water shall be deemed to comply with the South African Bureau of Standards' specification No. 241 - 1984 for water for domestic supplies as far as its bacterial content is concerned.

Report as > 300 per plate rather than TNTC (too numerous to count).

9. References

SMRI (1997). Determination of the bacteriological quality of water by the membrane filter method. *SMRI Test Methods*, TM200.

SABS 241 (1984). *South African Standard Specification for Water for Domestic Supplies*, June: 15 pp.

SABS (1986). Method 221, 1st revision, March, 7 pp.