



Method 8.18 – Refined sugar: total mesophilic bacteria, yeasts and moulds by the membrane filter method

1. Rationale

The method is used for the determination of the total mesophilic bacteria, yeasts and moulds in white sugar containing low numbers of organisms.

2. Principle

Test solutions of sugars are filtered through sterile membranes of known pore size. The organisms are retained on the membrane surface and can subsequently be detected as colony forming units after growing on a suitable nutrient medium or pad. Sterile glassware and aseptic technique must be used.

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 colony forming units (CFU) per 10 g of sample.

3.2 Mesophilic bacteria

Mesophilic bacteria refer to cellular microorganisms that have their growth optima between 20°C and 45°C. A general culture medium which favours the growth of a broad spectrum of bacteria is used.

3.3 Yeasts and Moulds

Yeasts and moulds are microorganisms that form colonies at 30°C. A selective medium with a low pH value (pH < 5) or in the presence of an antibiotic is chosen since it supports the growth of yeasts and moulds and inhibits the growth of bacteria.

4. Apparatus

4.1 Apparatus for dry sterilisation at 170 ± 5°C

This apparatus is used to sterilise glassware.

4.2 Autoclave for sterilisation at 121 ± 2°C

The autoclave is used for sterilisation of water and media.

4.3 Incubator operating at 30 ± 1°C

4.4 Filter funnel, base with support and clamp 50 mm ϕ , 100 cm³ capacity

The filtration apparatus construction material can be stainless steel, autoclavable plastic, glass or sterile disposable plastic.

4.5 Flask with side arm for mounting filter apparatus: 250 cm³

4.6 Vacuum pump

4.7 Pipettes: 10 cm³

4.8 Forceps

4.9 Colony counting equipment

4.10 Conical flasks: 500 cm³

4.11 Bunsen burner

5. Reagents

5.1 Sterile distilled water

Measure 100 cm³ distilled water into conical flasks, plug with cotton wool and cover with tin foil. Attach heat sensitive tape to the tin foil covering each flask. The tape will change colour to indicate a successful sterilisation cycle. Autoclave at 121 ± 2°C for 15 minutes.

5.2 Culture media

Sterile nutrient pad sets in Petri dishes with membrane filters of 0.45 µm pore size and 45 mm φ for bacteria.

Sterile nutrient pad sets in Petri dishes with membrane filters of 0.65 µm pore size and 45 mm φ for yeasts and moulds.

5.3 Disinfectant

Commercially available household disinfectant such as “Jik”.

5.4 Alcohol

Alcohol (ethanol, CH₃CH₂OH) is a flammable solvent and toxic and should be handled with care. Wear safety glasses to avoid contact with the eyes.

6. Procedure

6.1 Sample collection

Samples should be collected in a sterile container and should not be exposed to the atmosphere prior to analysis.

6.2 Preparation of the workplace

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

6.3 Medium preparation

Wet the nutrient pads with 3.0 - 3.5 cm³ of sterile distilled water. Ideally a slight excess of liquid should be visible at the edge of the pad.

6.4 Sample preparation

Mesophilic bacteria: add 1 g of crystalline sugar aseptically to a 500 cm³ conical flask containing 100 cm³ of sterile distilled water.

Yeasts and moulds: add 10 g of crystalline sugar aseptically to a 500 cm³ conical flask containing 100 cm³ of sterile distilled water.

Shake thoroughly to dissolve the sugar. Include one sterility check in every ten samples by using sterile distilled water instead of sample.

6.5 Filtration and incubation

Connect the filter apparatus to the vacuum pump. Place the membrane centrally on the base with sterile forceps. Place the sterile funnel on the top and clamp in place. 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 on a moistened nutrient pad. When positioning the membrane on the nutrient pad ensure that no air bubbles are trapped between them.

Incubate at 30 ± 1°C for 48 hours for mesophilic bacteria and for 72 hours for yeasts and moulds.

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

7. Expression of Results

7.1 Total mesophilic bacteria

Count the colonies on the membrane using the colony counting equipment. Multiply the result by ten and report as CFU per ten grams of sugar.

7.2 Yeasts and moulds

Count the colonies on the membrane using the colony counting equipment. Yeasts are opaque, white, yellow or pink, whilst moulds form a mycelium which is often white with black, brown or green spores. Report as CFU per ten grams of sugar.

If either result is more than 300 CFUs, report as > 300 CFU/10 g.

8. References

ICUMSA (1994). Total mesophilic bacterial count by the membrane filtration method. *ICUMSA Methods Book*, GS2/3-41.

ICUMSA (1994). Yeast and moulds. *ICUMSA Methods Book*, GS2/3-47.

SMRI (2004). Determination of the total mesophilic bacteria, yeasts and moulds in white sugar by the membrane filter method. *SMRI Test Methods*, TM202.