

PW02: Enumeration of total coliforms in tap water

This laboratory exercise outlines a standardized protocol for the enumeration of total coliforms in tap water samples employing the multiple-tube fermentation (MPN) procedure for estimating total coliforms in tap water using Brilliant Green Lactose Bile Broth (BGLB) with Durham tubes.

I. Principle

Total coliforms are operationally defined in fermentation-tube methods as bacteria that ferment lactose and produce gas under the specified test conditions. BGLB contains bile salts and brilliant green dye, which inhibit many non-coliform organisms while permitting the growth of coliforms.

II. Objective

- Detect and estimate total coliforms in tap water.
- Determine the MPN per 100 mL using McCrady tables

III. Materials and reagents

- Sterile sampling bottle.
- BGLB broth tubes with Durham tubes: Double-strength (2×) broth and Single-strength (1×) broth
- Sterile pipettes or micropipettes and sterile tips: 10 mL, 1 mL, 0.1 mL
- vortex mixer.

IV. Procedure

IV.1. Sampling :

- Disinfect the outlet (flame sterilization)
- Let the water run for 2–3 minutes to flush the line.
- Fill the bottle (leave a small headspace), cap immediately and label.

IV.2. Inoculation

- Arrange 9 tubes in three sets:
 - **Set A:** 3 tubes BGLB Double-strength: inoculate **10mL** sample into each tube
 - **Set B:** 3 tubes BGLB Single-strength: inoculate **1.0 mL** sample into each tube
 - **Set C:** 3 tubes BGLB Single-strength: inoculate **0.1 mL** sample into each tube
- Homogenize the sample by gentle inversion.
- Aseptically inoculate each tube with the correct volume.
- Mix gently (do not invert vigorously; avoid trapping air in Durham tubes).
- Incubate at 37°C
- Read at 24; if negative, continue incubation and read again at 48 h

IV.3. Reading

- Record each tube as:
 - Positive: gas present in Durham tube at the read time (and note turbidity if present)
 - Negative: no gas(Turbidity without gas is not considered a confirmed positive).
- Count the number of positive tubes in each set
- Use the McCrady MPN to obtain MPN.