A.2.1 MICROBIOLOGICAL PARAMETERS

A.2.1.1 Total Coli forms- Membrane Filtration Technique (APHA, 1994)

The Membrane Filtration (MF) method is a fast way to estimate bacterial populations in water. The MF method is especially useful when evaluating large sample volumes or performing many coli form tests daily. In the initial step, an appropriate sample volume is allowed to pass through a membrane filter with a pore size small enough (0.45 micron) to retain the bacteria present. The filter was placed on an absorbent pad (in a petri dish) saturated with a culture medium i.e., selective for coli form growth. The petri dish containing the filter and pad was incubated, upside down, for 24 hours at the appropriate temperature. After incubation, the colonies that have grown were identified and counted using a low power microscope. Pour Rite Ampules contained prepared selective media. This eliminated the measuring, mixing, and autoclaving needed for preparing dehydrated media. The ampules were designed with a large, unrestricted opening that allowed media to pour out easily. Each ampule contained enough medium for one test was used.

To test potable water with the MF Method, 100ml of the sample was examined for total coli forms by incubating a filter at 35 ± 0.5 °C for 22–24 hours on m-Endo Broth. Coli forms fermented lactose in the medium and produced an acid-aldehyde complex. This complex combined with Schiff’s Reagent (also in the medium) to form an iridescent green coating over the colonies. When magnified 10 to 15 times, coli forms appeared as dark red colonies with a greenish-gold sheen.
i. Presumptive Phase

- A sterile absorbent pad was placed in a sterile petri dish using sterilized forceps. The lid was replaced without touching the pad or the inside of the petri dish.
- An m-Endo Broth Pour Rite Ampule was inverted 2 to 3 times to mix the broth. Carefully, the contents were poured evenly over the absorbent pad. The petri dish lid was replaced. The steps 1 and 2 was repeated for each petri dish being prepared.
- The Membrane Filter Assembly was set-up and the sterilized forceps were used to place a membrane filter, grid side up, into the assembly.
- The sample was inverted to mix evenly for 30 seconds. 100 ml of sample was poured into the funnel by applying vacuum and filtered the sample. The vacuum was released and the funnel was rinsed with 20 to 30 ml of sterile buffered dilution water. Again vacuum was applied and the rinsing was repeated for two more times. The vacuum was released to prevent damage to the filter.
- The vacuum was turned off and the funnel top was lifted off using sterilized forceps and transferred immediately to the previously prepared petri dish.
- Checked for air trapped under the filter and made sure that the filter touches the entire pad and the petri dish lid was replaced.
- The petri dish was inverted and incubated at 35 ± 0.5 °C for 22–24 hours.
- After incubating, a 10 to 15X microscope was used to count the red colonies that have a greenish-gold metallic sheen. The sheen maybe extended over the entire colony, or it may be localized to the edge or to the center.
- The results were recorded.
ii. Confirmation phase

The water samples, confirmed typical colonies to ensure they are coli forms. (Confirmed with sheen colonies, up to a maximum of five.) Inoculated parallel tubes of Lauryl Tryptose (LT) single-strength (SS) Broth and Brilliant Green Bile (BGB) Broth by transferring growth from each colony. Growth and gas production in both tubes verified that the suspect organisms are coli forms. Most Probable Number (MPN) coli form tubes are ideal for this purpose.

- Asterilised needle was used to transfer from the coli form (sheen) colony grown on m-Endo plate to a single strength Lauryl Tryptose (LT) Broth tube.
- Again the same coli form colony was touched with the needle and transferred to a Brilliant Green Bile (BGB) Broth tube.
- Both tubes were inverted to eliminate any air bubbles trapped in the inner vials. Incubated the tubes at 35 ± 0.5 °C. After one hour, the tubes were inverted to remove trapped air in the inner vial, followed by incubation.
- After 24 ± 2 hours, the inner vials were checked for growth and gas bubbles. Growth (turbidity) and gas bubbles in both the LT and BGB Broth tubes verified that the colonies were coli forms. (If one or both tubes do not show gas, continue incubating both tubes for an additional 24 hours.) If no gas is present in the LT Broth tube after 48 hours, the colony is not a coli form and additional testing is unnecessary).
- The results were recorded.
A.2.1.2 Fecal Coli forms

To analyze fecal coli forms in total-coli form-positive potable water samples a confirmation test was done.

The membrane was swabbed with a sterile cotton swab and inoculated into a tube of EC Medium Broth. Growth and gas production in the EC Medium after incubation at 44.5 ± 0.2 °C for 24 hrs, confirmed the presence of fecal coli forms.

A.2.1.3 Interpreting and Reporting Results

The coli form density as the number of colonies per 100 mL of sample was reported for both total coli forms and fecal coli forms. The samples that produced 20 to 80 coli form colonies, and not more than 200 colonies of all types, per membrane were used to compute coli form density.

For fecal coli form testing, samples should produce 20 to 60 fecal coli form colonies. Equation A was used to calculate coli form density. Note that “mL sample” refers to actual sample volume, and not volume of the dilution.

Equation A—Coli form density on a single membrane filter

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\text{Coli form colonies per 100 ml} = \frac{\text{Coli form colonies counted}}{\text{ml of sample filtered}} \times 100
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Thus the total coli forms and fecal coli forms were calculated and tabulated.