Compendium of Indian Standards for

Detection of Fecal Indicators in Packaged Water

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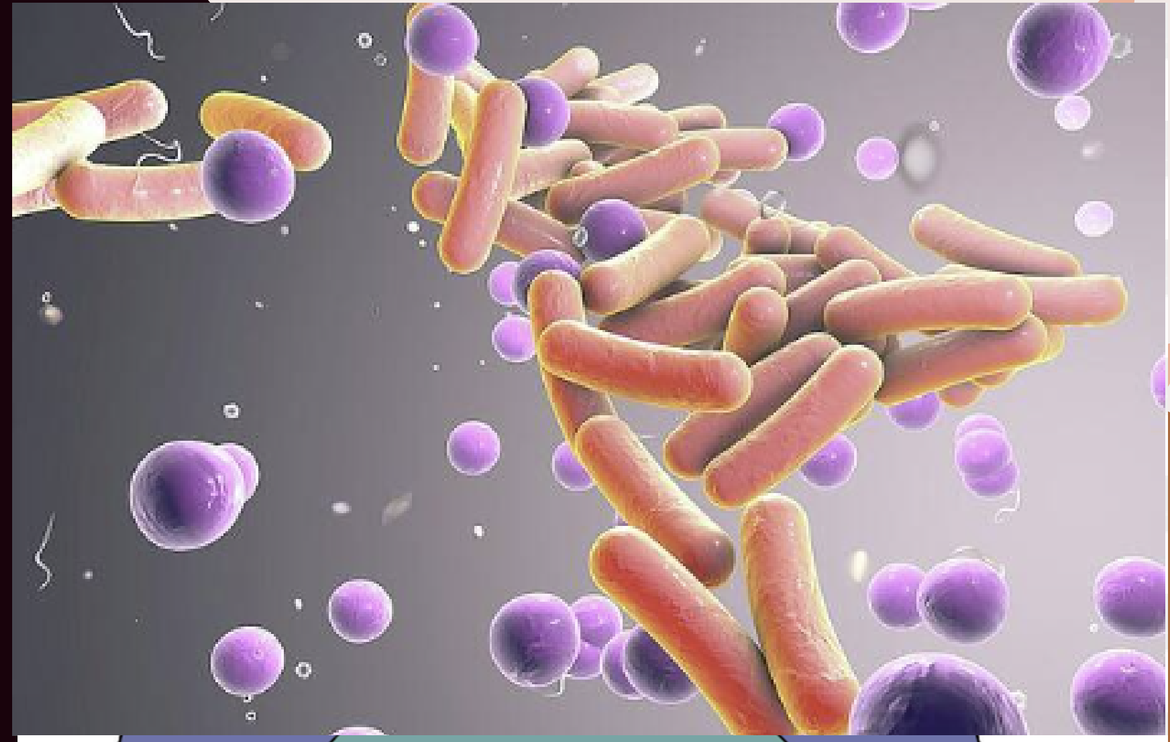

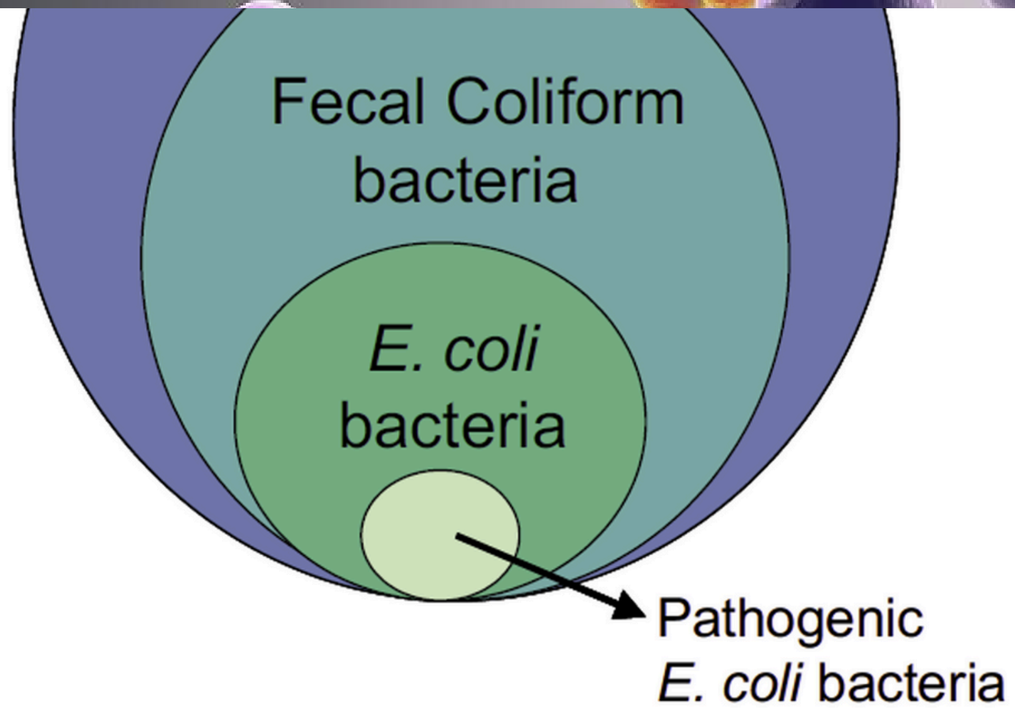

Table of Contents

1. Introduction

Fecal indicators remain a valuable tool for assessing water quality and have been expanded to include markers capable of detecting sources of faecal contamination. Faecal indicator bacteria have been employed for over 150 years to indicate fecal contamination in water and the associated health risks.

Sewage contamination spreads diseases such as cholera and typhoid created the need for reliable methods to detect sewage in drinking water. Coliform bacteria—a group of generally harmless Gram-negative bacteria naturally found in the gastrointestinal tracts of humans and other warm-blooded animals—provided a simple and relatively reliable tool for identifying sewage pollution due to their high concentration in sewage and ease of cultivation.

Faeces may contain a wide range of pathogens, which upon entering the environment can persist for varying durations, often at concentrations too low for reliable detection, yet sufficient to pose health risks. Additionally, conventional methods for detecting enteric pathogens are typically time-consuming, expensive, and often lack sensitivity, even in fresh faecal samples. Therefore, faecal indicator bacteria (FIB), such as the faecal coliform *Escherichia coli*, continue to serve as effective indicators of hazardous faecal contamination in water.

The presence of faecal indicators in packaged water represents a critical measure of microbiological quality. Key fecal indicators include Coliforms, *Escherichia coli*, *Streptococcus faecalis* (Enterococci), and Sulphite-Reducing Anaerobes. Detection of these organisms suggests potential fecal contamination and indicates the possible presence of pathogenic microorganisms. Indian Standards prescribe validated methodologies for detecting these faecal indicators.

2. List of products specifying requirements for Fecal Indicator Bacteria:

A. Packaged Drinking Water as per IS 14543 & Packaged Natural Water as per IS 13428:

Parameter	Requirement
Coliform in 250 ml sample	Absent
Escherichia coli or thermotolerant bacteria in 250 ml sample	Absent
Faecal streptococci in 250 ml sample	Absent
Sulphite-Reducing Anaerobes in 50 ml sample	Absent

B. Drinking Water as per IS 10500:

Parameter	Requirement
E. coli or thermotolerant coliform bacteria in 100 ml sample	Absent
Total coliform bacteria in 100 ml sample	Absent

3. Test Method Standards:

• **IS 15185: 2016** Water Quality — Detection and enumeration of *Escherichia Coli* and coliform bacteria — Membrane Filtration Method for water with low bacterial background flora

• **IS 15186: 2002** Water Quality — Detection and enumeration of intestinal Enterococci — Membrane Filtration Method

• **IS 5401 (Part 1): 2012** Microbiology of Food And Animal Feeding Stuffs — Horizontal Method for the detection and enumeration of Coliforms Part 1 Colony-Count Technique

• **Annex C of IS 13428: 2024** Detection and enumeration of the spores of Sulphite-Reducing Anaerobes (Clostridia)

4. IS 15185 – Water Quality — Detection and enumeration of *Escherichia coli* and Coliform bacteria — Membrane Filtration Method for water with low bacterial background flora

Scope:

This method specifies procedures for the enumeration of *Escherichia coli* (*E. coli*) and coliform bacteria. The technique involves membrane filtration, subsequent cultivation on Chromogenic Coliform Agar medium, and enumeration of the target organisms present in the sample. Due to the limited selectivity of this differential agar medium, background microbial growth may interfere with accurate enumeration of *E. coli* and coliform bacteria, particularly in surface waters or shallow wells; thus, the method is not suitable for these water types.

Certain strains of *E. coli*, notably β-D-glucuronidase-negative strains like *Escherichia coli* O157, will not be detected as *E. coli*. Since these strains exhibit β-D-galactosidase activity, they will appear as coliform bacteria on the chromogenic agar medium.

Principle

The method involves filtering a measured sample volume through a membrane filter, which retains the microorganisms. This membrane is then placed onto a Chromogenic Coliform Agar plate and incubated at (36 ± 2) °C for (21 ± 3) hours. Colonies exhibiting β-D-galactosidase activity (pink to red coloration) are counted as presumptive coliform bacteria (excluding E. coli). To prevent false-positive results caused by oxidase-positive bacteria, such as *Aeromonas* spp., presumptive coliform colonies should be confirmed by performing an oxidase test (negative reaction). Colonies demonstrating both β-D-galactosidase and β-D-glucuronidase activity (dark blue to violet coloration) are counted as *E. coli*. The total coliform count is calculated as the sum of oxidase-negative colonies with pink-to-red color and all dark blue-to-violet colonies.

Filtration

Filter 250 ml of the sample using a sterile membrane filter.

Incubation and Differentiation

After filtration, carefully place the membrane filter onto a Chromogenic Coliform Agar (CCA) plate. Ensure no air bubbles are trapped underneath. Invert the Petri dish and incubate it at (36 ± 2) °C for (21 ± 3) hours.

After incubation:

Count all colonies exhibiting a positive β-D-galactosidase reaction (pink to red colonies) as **presumptive coliform bacteria (excluding *E. coli*).**

Count all colonies exhibiting both β-D-galactosidase and β-D-glucuronidase reactions (dark blue to violet colonies) as ***E. coli*.**

To confirm presumptive coliform colonies (pink to red colonies, not identified as *E. coli*), perform **an oxidase test**.

Observe color change: oxidase-positive colonies will produce a dark blue color within 30 seconds. True coliform bacteria should yield a negative oxidase reaction (no color change).

5. IS 15186: Water Quality — Detection and enumeration of intestinal Enterococci — Membrane Filtration Method

Scope

This standard specify method for the detection and enumeration of intestinal enterococci in water samples using the membrane filtration technique. This standard is primarily designed for testing drinking water, swimming pool water, and other disinfected or relatively clean waters. It is especially suitable for analyzing large volumes of water expected to contain relatively few intestinal enterococci.

Principle

Filtration, Incubation, and Enumeration

The enumeration of intestinal enterococci is conducted by filtering a 250 ml volume of water through a membrane filter (0.45 µm pore size), which effectively retains the target bacteria. The membrane is then placed onto a selective agar medium containing sodium azide, which inhibits the growth of Gram-negative bacteria, and 2,3,5-triphenyltetrazolium chloride (TTC), a colorless dye reduced to red formazan by intestinal enterococci. Incubate the plates at (36 ± 2) °C for (44 ± 4) hours.

After incubation, typical colonies of enterococci appear raised with a red, maroon, or pink color, either at the colony's center or distributed throughout.

Confirmation

After incubation, identify typical colonies that are raised and appear red, maroon, or pink, either in the center or throughout the colony. To confirm these colonies, transfer the membrane (without inversion) using sterile forceps onto a Bile-Aesculin-Azide Agar (BEAA) plate preheated to 44°C. Incubate at (44 ± 0.5) °C for 2 hours.

Intestinal enterococci hydrolyze aesculin within approximately 2 hours on this medium, producing an end product (6,7-dihydroxycoumarin) which reacts with iron(III) ions, resulting in a tan-colored to black precipitate diffusing into the medium. Colonies surrounded by a black or dark tan coloration are thus confirmed as intestinal enterococci.

6. IS 5401 (Part 1): Microbiology of Food and Animal Feeding Stuffs — Horizontal Method for the detection and enumeration of Coliforms Part 1 Colony-Count Technique

Scope

This Standard provides general guidelines for the enumeration of coliform bacteria. It applies to:

- Products intended for human consumption and animal feeding, and
- Environmental samples collected from food production and food handling areas.

The method involves counting colonies after incubation on a solid selective medium.

Principle

This method involves enumeration of coliform bacteria by a colony-count technique using Violet Red Bile Lactose (VRBL) Agar, followed by confirmation in Brilliant Green Lactose Bile Broth (BGLB). Coliform bacteria ferment lactose present in VRBL Agar, resulting in acid production. This acidification causes colonies to appear purplish-red, often surrounded by a characteristic zone of precipitated bile. Suspected colonies are further confirmed by inoculating into BGLB broth, where gas production within Durham tubes confirms their identification as coliform bacteria.

7. Annex C of IS 13428: Detection and enumeration of the spores of Sulphite-Reducing Anaerobes (Clostridia)

Principle

The spores of sulphite-reducing anaerobes (clostridia) are widespread in the environment. They are present in human and animal faecal matter, in waste water and, in soil. Unlike *Escherichia coli* and other coliform organism, the spores survive in water for long periods as they are more resistant than vegetative forms to the action of chemical and physical factors. They may thus give an indication of remote or intermittent pollution. They may even be resistant to chlorination at levels which are normally used for the treatment of water.

Procedure

Detection by anaerobic growth in double strength Differential Reinforced Clostridial Medium (DRCM), producing black precipitate. Sample heated (75–80°C, 15 min) to activate germination of spores, incubated anaerobically at 37°C ± 1°C for 44 ± 4 hours.

8. Conclusion

This compendium compiles the essential Indian Standards and test protocols for the detection of faecal indicators in packaged water. The outlined principles, media, procedures, and confirmation steps ensure regulatory compliance and consumer safety.