M-E.coli Broth

M1426

Intended Use:

Recommended for the detection, differentiation and enumeration of *Escherichia coli* and coliforms in water samples using membrane filter technique without further confirmation.

Composition**

Ingredients	g / L
Tryptone	20.000
Bile salts mixture	1.500
Chromogenic mixture	0.175
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 21.67 gram in 1000 ml purified/ distilled water. Heat if necessary to dissolve the medium completely. **DO NOT AUTOCLAVE.** Cool to 45-50°C, aseptically add desired quantity (2 to 5 ml) of broth on sterile absorbent cotton pad for saturation. The medium should be used within 24 hours of rehydration.

Principle And Interpretation

M-E.coli Broth is used for detection and differentiation of *Escherichia coli* and coliforms in water samples using membrane filter technique. It is based on Tryptone Bile Agar used for detection of *Escherichia coli* in foods (1) where recovery of *Escherichia coli* is faster, more reliable and accurate.

The water sample is filtered through membranes and then placed on pad saturated with M-E.coli Broth and incubated at 37° C in sealed Petri plates. The medium contains chromogenic mixture, which helps to detect glucuronidase activity of *Escherichia coli* (2). This specific enzyme differentiates *Escherichia coli* from other coliforms. *Escherichia coli* cells split the chromogenic mixture with the help of glucuronidase to give blue colouration to the colonies. Coliforms other than *Escherichia coli* turn red as they reduce TTC (2,3,5-triphenyl tetrazolium chloride). Thus, the resulting colour distinction allows simple interpretation of test without further confirmation. Tryptone provides the essential growth nutrients to the organisms. Bile salts inhibit gram - positive organisms.

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. ß-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
2. Some species may show poor growth due to nutritional variations.
3. Further biochemical and serological test are necessary for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to beige homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured, clear solution without any precipitate

Reaction

Reaction of 2.16 % w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours

Organism	Inoculum (CFU)	Growth	Colour of Colony
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant	red
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	blue
Staphylococcus aureus subsp *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	

Key : (*) Corresponding WDCM numbers. (#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Anderson J. M. and Baird Parker A.C., (1975), J. Appl. Bact., 39:111.
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Hansen W. and Yourassawsky E., (1984), J. Clin. Microbiol. 20:1177.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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