EC Broth

M127I

Intended Use:

Recommended for selective enumeration of presumptive *Escherichia coli* by MPN technique. The composition and performance criteria of this medium are as per the specifications laid down in ISO/DIS 7251:2005 Amd.1:2023 (E) and ISO 11133:2014 / Amd. 2 : 2020 (E) .

Composition**

ISO Specification - EC Broth (Selective Medium)		**M127I - EC Broth**	
Ingredients	**g / L**	**Ingredients**	**g / L**
Enzymatic digest of casein	20.000	Tryptone #	20.000
Lactose	5.000	Lactose	5.000
Bile salts No. 3	1.500	Bile salts mixture ##	1.500
Potassium monohydrogen phosphate (K2HPO4)	4.000	Dipotassium hydrogen phosphate	4.000
Potassium dihydrogen phosphate (KH2PO4)	1.500	Potassium dihydrogen phosphate	1.500
Sodium chloride	5.000	Sodium chloride	5.000
Final pH (at 25°C)	6.8±0.2	Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Key : # - Equivalent to Enzymatic digest of casein, ## - Equivalent to Bile salts No. 3

Directions

Suspend 37.0 gram in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense in test tubes containing inverted Durhams tube. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Adjust the concentration of medium in accordance with sample size.

Principle And Interpretation

EC Medium is used for detection of coliforms during bacteriological examination of water, milk and foods. It was originally described by Hajna & Perry (1). This medium was later used by Fishbein and Surkiewicz to carry out *Escherichia coli* confirmatory tests (2). It is also used in MPN methods (3) and is often used for confirmation of coliforms. The procedure employing EC Medium provides information regarding the source of the coliform group (fecal or non-fecal) when used as a confirmatory test (4). It should not be used for the direct isolation of coliforms since prior enrichment in a presumptive medium for optimal recovery of fecal coliforms is required. The medium is as per specifications laid down in ISO (5,6)

Tryptose provides essential growth nutrients. Lactose is the fermentable sugar. Bile salts mixture inhibit gram-positive bacteria especially bacilli and faecal Streptococci. Potassium phosphates control the pH during fermentation of lactose. Gas production in a fermentation tube within 24 hour or less is a presumptive evidence of the presence of coliform bacteria. This medium can be used at 37°C for the detection of coliform organisms or at 44.5°C for the isolation of *Escherichia coli* from water and shellfish) or 45.5°C for foods

When using sample more than 10 ml, the medium must be reconstituted at a concentration equivalent to that specified on the directions, once the sample is added, the working procedure is as follows. Transfer a loopful of culture from all the tubes of Lauryl Sulphate Broth (M080) showing gas formation within 24 hours and from all the tubes showing xbacterial growth within 48 hours to EC Broth tubes. Within 30 minutes from the inoculum, place the tubes in a water bath and incubate at 44°C for 24 hours. Consider the growth showing gas production as positive. Calculate the density of the faecal coliform organisms by using MPN tables. False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents (7).

Gas formation at 44.5°C or 45.5°C (and 37°C) Gas formation at 37°C	*Escherichia coli*, possibly also other coliforms. Coliform bacteria without *Escherichia coli*

Type of specimen

Food samples - Food and animal feeding stuffs

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6).
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. False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents (6).

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

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured, clear solution without any precipitate

Reaction

Reaction of 3.7% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Productivity : Cultural characteristics observed after an incubation at 44 ± 1°C for 24 ± 2 to 48 ± 2 hours.

Selectivity : Cultural characteristics observed after an incubation at 44 ± 1°C for 24 ± 2 to 48 ± 2 hours

Organism	Inoculum (CFU)	Growth	Gas production
Productivity			
Escherichia coli ATCC 25922 (00013*)	50-100	good	positive reaction
Escherichia coli ATCC 8739 (00012*)	50-100	luxuriant	positive reaction
Selectivity			
Pseudomonas aeruginosa ATCC 27853 (00025*)	>=10^4	inhibited	

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°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 (8,9).

Reference

1. Hajna A. A. and Perry C. A., 1943, Am. J. Public Health, 33:550.
2. Fishbein M. and Surkiewicz B. F., 1964, Appl. Microbiol., 12:127.
3. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

4. Marshall, (Ed.), 1993, Standard Methods for the Examination of Dairy Products, 16th Ed., American Public Health Association, Washington, D.C.
5. Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique. ISO/DIS 7251:2005 & Amd.1:2023(E)
6. Microbiology of food,animal feeding stuffs and water- Preparation, production,storage and performance culture media, ISO 11133:2014 /Amd. 2 : 2020 (E) .
7. Ray B., 1986, J. Food Prot., 49:651.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. 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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