EC Broth

M127

EC Broth is recommended for the selective enumeration of presumptive *Escherichia coli* by MPN technique.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Lactose	5.000
Bile salts mixture	1.500
Dipotassium phosphate	4.000
Monopotassium phosphate	1.500
Sodium chloride	5.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37 grams in 1000 ml 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. 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 and 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). EC Broth 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.

Casein enzymic hydrolysate provides essential growth nutrients. Lactose is the fermentable sugar. Bile salts mixture inhibit gram-positive bacteria especially bacilli and faecal Streptococci. 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 (5).

Gas formation at 44.5°C *Escherichia coli* , possibly

or 45.5°C (and 37°C), also other coliforms.

Gas formation at 37°C Coliform bacteria without Escherichia coli

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 : 6.9±0.2

pH
6.70-7.10

Cultural Response
Cultural characteristics observed after an incubation at 44.5°C ± 0.2 for 24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Gas
Cultural Response			
Bacillus subtilis ATCC 6633	>=10^3	inhibited	
Escherichia coli ATCC 25922	50-100	good-luxuriant	positive reaction
Enterobacter aerogenes ATCC 13048	>=10^3	inhibited	
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	
Klebsiella pneumoniae ATCC 13883	50-100	good-luxuriant	positive reaction
Pseudomonas aeruginosa ATCC 27853	50-100	fair to good	negative reaction

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

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. Eaton A. D., Clesceri L. S. and Greenberg A. E., (Ed.), 1998, Standard Methods for the Examination of Water and Waste water, 20th Ed., American Public Health Association. Washington, D.C.
4. Marshall, (Ed.), 1993, Standard Methods for the Examination of Dairy Products, 16th Ed., American Public Health Association, Washington, D.C.
5. Ray B., 1986, J. Food Prot., 49:651.

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