EMB Broth

M503

Intended Use:

Recommended for the differentiation of Gram-negative enteric bacteria from clinical & non-clinical specimens.

Composition**

Ingredients	g / L
Peptone	10.000
Lactose	5.000
Sucrose	5.000
Dipotassium hydrogen phosphate	2.000
Eosin - Y	0.400
Methylene blue	0.065
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 22.46 grams in 1000 ml purified/distilled water. Mix until suspension is uniform. Heat if necessary to dissolve the medium completely. Dispense in tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. **AVOID OVERHEATING.** Cool to 45-50°C and shake the medium in order to oxidize the methylene blue (i.e. to restore its blue colour) and to suspend the flocculent precipitate.

Precaution:*Store the medium away from light to avoid photooxidation.*

Principle And Interpretation

Eosin Methylene Blue (EMB) media were originally devised by Holt-Harris and Teague (1) and further modified by Levine (2). The above media are combination of the Levine and Holt-Harris and Teague formulae which contains peptic digest of animal tissue and phosphate as recommended by Levine and two carbohydrates as suggested by Holt-Harris and Teague. EMB Broth has a similar composition as EMB Agar except agar.

Methylene blue and Eosin-Y inhibit gram-positive bacteria to a limited degree. These dyes serve as differential indicators in response to the fermentation of carbohydrates. The ratio of eosin and methylene blue is adjusted approximately to 6:1. Sucrose is added to the medium as an alternative carbohydrate source for typically lactose-fermenting, gram-negative bacilli, which on occasion do not ferment lactose or do so slowly. The coliforms produce purplish black broth due to taking up of methylene blue-eosin dye complex, when the pH drops. Nonfermenters probably raise the pH of surrounding medium by oxidative deamination of protein, which solubilizes the methylene blue-eosin complex resulting in colourless broth (3). Some strains of *Salmonella* and *Shigella* species do not grow in the presence of eosin and methylene blue. Peptone serves as source of carbon, nitrogen, and other essential growth nutrients. Lactose and sucrose are the sources of energy by being fermentable carbohydrates. Eosin-Y and methylene blue serve as differential indicators. Dipotassium hydrogen phosphate buffers the medium.

Type of specimen

Clinical samples- Faecal samples, Food samples, Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5). For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. Further biochemical and serological tests must be carried out for complete identification.

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 pink to purple homogeneous free flowing powder

Colour and Clarity of prepared medium

Reddish purple coloured, opalescent solution with greenish cast and finely dispersed precipitate in tubes

Reaction

Reaction of 2.25% 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 medium
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	Purple with green metallic sheen
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	good	pink
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good	pink
Proteus mirabilis ATCC 25933	50-100	luxuriant	colourless
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	colourless
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 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Holt-Harris and Teague,1916, J. Infect. Dis., 18 : 596.
2. Levine, 1918, J. Infect. Dis., 23:43.
3. Howard B.J., 1994, Clinical and Pathogenic Microbiology, 2nd ed., Mosby Year Book, Inc
4. Isenberg (Eds.), 1992, Clinical Microbiology Procedures Handbook, Vol . 1, American Society for Microbiology, Washington, D.C.
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.
6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

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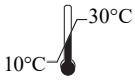
HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

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