



EMB Broth

M503

EMB Broth (Eosin Methylene Blue Broth) is recommended for the differentiation of gram-negative bacteria from clinical and nonclinical specimens.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Lactose	5.000
Sucrose	5.000
Dipotassium 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 distilled water. Mix until suspension is uniform. Heat if necessary to dissolve the medium completely. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. AVOID OVERHEATING. Cool to 50°C and shake the medium in order to oxidize the methylene blue (i.e. to restore its blue colour) and to suspend the flocculant 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. Further tests are required for confirmation. Peptic digest of animal tissue 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. Phosphate buffers the medium.

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

M503: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth	Colour of medium
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	Purple with green metallic sheen
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	good	pink
<i>Klebsiella pneumoniae</i> ATCC 13883	50-100	good	pink
<i>Proteus mirabilis</i> ATCC 25933	50-100	luxuriant	colourless
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant	colourless
<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	inhibited	

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. 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.

Revision : 1 / 2011



Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.