Technical Information

EMB Broth

Product Code: DM1503

Application: - 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

Principle & Interpretation

Eosin Methylene Blue (EMB) media are combination of the Levine [1] and Holt-Harris and Teague [2] 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 the growth of gram-positive bacteria to a limited degree. These dyes also act 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 their 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.

Methodology

Suspend 22.46 grams of powder media in 1000 ml distilled water. Mix until suspension is uniform. Shake well & heat 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.

Quality Control

Physical 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 range 7.00-7.40

Cultural Response/ characteristices
Dm 1503: 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	50-100	luxuriant	Purple with green metallic sheen
Enterobacter aerogenes ATCC 13048	50-100	good	Pink
Klebsiella pneumoniae ATCC 13883	50-100	good	Pink
Proteus mirabilis ATCC 25933	50-100	luxuriant	Colourless
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	Colourless
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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.

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