EMB Agar

M317

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

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Dipotassium phosphate	2.000
Lactose	5.000
Sucrose	5.000
Eosin - Y	0.400
Methylene blue	0.065
Agar	13.500
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.96 grams in 1000 ml distilled water. Mix until suspension is uniform. Heat to boiling to dissolve the medium completely. 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. (If EMB Agar is inoculated on the same day, it may be used without autoclave sterilization).

Precaution : Store the medium away from light to avoid photooxidation

Principle And Interpretation

Eosin Methylene Blue (EMB) Agar was originally devised by Holt-Harris and Teague (1) and further modified by Levine (2). The above medium is a 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.

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 colonies due to taking up of methylene blue-eosin dye complex, when the pH drops. The dye complex is absorbed into the colony. Nonfermenters probably raise the pH of surrounding medium by oxidative deamination of protein, which solubilizes the methylene blue-eosin complex resulting in colourless colonies (3). Some strains of *Salmonella* and *Shigella* species do not grow in the presence of eosin and methylene blue. Further tests are required to confirm the isolates.

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.

The test sample can be directly streaked on the medium plates. Inoculated plates should be incubated, protected from light. However standard procedures should be followed to obtain isolated colonies. A non-selective medium should be inoculated in conjunction with EMB Agar. Confirmatory tests should be further carried out for identification of isolated colonies.

Quality Control

Appearance

Light pink to purple homogeneous free flowing powder

Gelling

Firm, comparable with 1.35% Agar gel.

Colour and Clarity of prepared medium
Reddish purple coloured, opalescent gel with greenish cast and finely dispersed precipitate forms in Petri plates

Reaction
Reaction of 3.6% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
M317: Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterobacter aerogenes ATCC 13048	50-100	good	40-50%	pink, without sheen
Escherichia coli ATCC 25922	50-100	luxuriant	>=50%	purple with black centre and green metallic sheen
Klebsiella pneumoniae ATCC 13883	50-100	good	40- 50%	pink, mucoid
Proteus mirabilis ATCC 25933	50-100	luxuriant	>=50%	colourless
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	>=50%	colourless
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2-8°C and away from the light. 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

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