

Technical Information

E M B Agar

Product Code: DM1317

Application: - E M B 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

Principle & 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.

Beside inhibitory growth of gram-positive bacteria to a limited degree by Methylene blue and Eosin-Y dyes they 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 some times fail to ferment lactose or do so slowly when the pH drops the dye complex is absorbed into the colony which appears purplish black 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. Additional 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.

Methodology

Suspend 35.96 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 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

Quality Control

Physical 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 range 7.00-7.40

Cultural Response/ characteristics

Dm 1317: Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours

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

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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