Technical Information

EMB Agar Plate

Product Code: PM 1317

Application: - Recommended for differential isolation of Gram-negative enteric bacilli from clinical and non clinical specimens.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Dipotassium hydrogen phosphate	2.000
Lactose	5.000
Saccharose (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 (5). The above medium is a combination of the Levine and Holt-Harris and Teague formulae which contains peptone 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 (2). 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. 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. 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.

Type of specimen

Clinical samples - Clinical samples- Faecal samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. Read the label before opening the pack. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling 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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Confirmatory tests should be further carried out for identification of isolated colonies.
4. Some strains of Salmonella and Shigella species do not grow in the presence of eosin and methylene blue.
5. It is recommended to store the plates ta 24-30°C to avoid minimum condensation.

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

Methodology

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Quality Control

Appearance
Light pink to purple homogeneous free flowing powder
pH
7.00-7.40
Gelling
Firm, comparable with 1.35% Agar gel.
Quantity of medium
25 ml of medium in 90 mm disposable plates.
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
Cultural Response
Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours

Oragnism	Inoculum (CFU)	Growth	Recovery	Color of colony
# *Klebsiella aerogenes* ATCC 13048(00175*)	50-100	good	40-50%	pink, without sheen
Escherichia coli ATCC 25922(00013*)	50-100	luxuriant	>=50%	purple with black centre and green
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good	40- 50%	pink, mucoid
Proteus mirabilis ATCC 25933	50-100	luxuriant	>=50%	colourless
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	>=50%	colourless
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : (*) Corresponding WDCM numbers. (#) Formerly known as Enterobacter aerogenes

Storage and Shelf Life

- On receipt store between 20-30°C.
- Use before expiry date on the label.
- 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 (5,6).

Further Reading

1.Holt-Harris and Teague,1916, J. Infect. Dis., 18 : 596.
2. Howard B.J., 1994, Clinical and Pathogenic Microbiology, 2nd ed., Mosby Year Book, Inc.
3. Isenberg (Eds.), 1992, Clinical Microbiology Procedures Handbook, Vol . 1, American Society for Microbiology, Washington, D.C.
4. 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.
5.Levine, 1918, J. Infect. Dis., 23:43.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and developmentwork carried at **CDH** is true and accurate
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