EMB Agar Plate

MP317

Intended Use:

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

Composition**

Ingredients	g/ L
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

Directions

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

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 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 (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.

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- Faecal samples, Food samples, Water samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (4).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (5).

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use. For professional use only. 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 at 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.

Quality Control

Appearance

Sterile EMB Agar in 90 mm disposable plates with smooth surface and absence of black particles/cracks/bubbles

Colour of medium

Reddish purple coloured medium with green cast

Quantity of medium

25 ml of medium in 90 mm disposable plates.

pH

7.00-7.40

Sterility Check

Passes release criteria

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour 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 metallic sheen
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*)	>=10^3	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 (6,7).

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.
4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
6. Isenberg (Eds.), 1992, Clinical Microbiology Procedures Handbook, Vol . 1, American Society for Microbiology, Washington, D.C.
7. 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.

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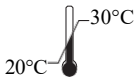
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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www.cepartner4u.eu

CE Marking

Do not use if package is damaged

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