ARENA BioScien

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EMB Agar

For the selective and differentiation of Gram-negative enteric bacteria from clinical and non-clinical specimens.

REF: BS.1/DB01.100.0100	100 Gram	REF: BS.1/DB01.250.0250	250 Gram
REF: BS.1/DB01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE

Eosin Methylene Blue (EMB) Agar was originally developed by Holt-Harris and Teague (1) and was subsequently modified by Levine (2). This formulation combines the features of both media by incorporating peptone and phosphate as recommended by Levine, together with lactose and sucrose as the fermentable carbohydrates proposed by Holt-Harris and Teague. Eosin Y and methylene blue act as selective agents by partially inhibiting the growth of Gram-positive bacteria while also functioning as differential indicators of carbohydrate fermentation. The dyes are maintained at an approximate eosin-to-methylene blue ratio of 6:1 to optimize colony differentiation. The inclusion of sucrose provides an additional fermentable carbohydrate, allowing the detection of lactose-fermenting Gram-negative bacilli that ferment lactose slowly or inconsistently.Fermentation of lactose and/or sucrose results in acid production, lowering the pH and promoting uptake of the eosin-methylene blue dye complex by the colonies. Consequently, coliform organisms typically produce dark purple to black colonies, often with a characteristic metallic green sheen in strong fermenters. In contrast, non-fermenting organisms oxidatively deaminate peptones, increasing the pH of the surrounding medium. This process dissolves the dye complex, resulting in colourless or translucent colonies (3). Certain strains of Salmonella and Shigella may exhibit reduced or no growth on EMB Agar because of their sensitivity to eosin and methylene blue; therefore, additional confirmatory tests are required for definitive identification. Peptone supplies carbon, nitrogen, vitamins, and other essential nutrients necessary for bacterial growth. Lactose and sucrose serve as fermentable carbohydrate sources, while eosin Y and methylene blue function as both selective and differential agents. Phosphate acts as a buffering system to maintain the stability of the medium. Test specimens should be streaked directly onto the surface of EMB Agar plates and incubated under appropriate conditions while protected from light. Standard microbiological techniques should be followed to obtain well-isolated colonies. For optimal recovery of susceptible organisms, a non-selective medium should be inoculated in parallel with EMB Agar. Presumptive isolates should be subjected to appropriate biochemical and/or serological confirmatory tests for final identification.

MEDIA COMPOSITION

Item	- Formula per - liter of medium
- Peptone	10.0
- Dipotassium hydrogen phosphateDextrose	2.0
- Lactose	5.0
- Sucrose	5.0
- Eosin -Y	0.400
- Methylene blue	0.065
- Agar	13.5

Final pH 7.2 ± 0.2 at 25°C

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves,

glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental Contamination.
S61: avoid release in environment.
For further information, refer to the EMB AGAR material safety data sheet.

STORAGE AND STABILITY

BioScien Eosin Methylene Blue (EMB) Agar dehydrated media are stable until expiration date stated on label when properly stored 10-30°C. The prepared medium should be stored at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

MEDIA PREPARATION

Suspend 35.96 grams in 1000 ml purified / 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.

Deterioration

The color of BioScien EMB Agar is Light pink to purple homogeneous free flowing powder. If there are any physical changes, discard the medium. Colour and Clarity of prepared medium Reddish purple coloured, opalescent gel with greenish cast and finely dispersed precipitate forms in Petri plates.

SPECIMEN COLLECTION AND PRESERVATION

Food samples ; Water and sewage samples

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile cups
- Sterile tubes
- Sterile loops
- Incubator

PERFORMANCE CHARACTERISTICS

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

Test Organisms	Growth	Colour of colony
Escherichia coli ATCC 25922	Luxuriant	purple with black Centre and green metallic sheen
Klebsiella pneumoniae ATCC 13883	Good	*Pink, Mucoid*
Salmonella Typhimurium ATCC 14028	luxuriant	Colorless
Staphylococcus aureus subsp. aureus ATCC	inhibited	-
Proteus mirabilis ATCC 25933	luxuriant	Colorless

SYMBOLS IN PRODUCT LABELLING	
IVD For in-vitro diagnostic use	Number of <n> test in the pack
LOT Batch Code/Lot number	Caution
REF Catalogue Number	Do not use if package is damaged
Temperature Limitation	Consult Instruction for use
Expiration Date	
Manufactured by	

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact ***BioScien*** technical support.

REFERENCES

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.