Eosin Methylene Blue Agar (EMB)
Cat #: MM-M-N039
For the isolation, cultivation and differentiation of Gram negative enteric bacilli from clinical and other specimens

Principles and uses:

Eosin Methylene Blue Agar (EMB) is a differential medium similar to Levine EMB Agar (Cat. MM-M-050), used for the isolation of Enterobacteria. The use of eosin Y and methylene blue enable differentiation between lactose-fermenting and non-fermenting organisms. It is widely used in medical bacteriology, in techniques recommended by APHA and for the detection and enumeration of coliforms, contaminants of foods and drinking water.

Peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Sucrose is added to Lactose as a fermentable carbohydrate to detect coliforms that ferment sucrose more readily than lactose. Eosin Y and methylene blue dyes are both partial inhibitors of Gram-positive bacteria and pH indicators. Due to the lactose and sucrose, this medium can be differential in primary culture: Salmonellae and Shigellae which are lactose-negative can be differentiated from other lactose-negative and sucrose-positive organisms such as Proteus vulgaris, Citrobacter and Aeromonas. Dipotassium phosphate acts as a buffer system and bacteriological agar is the solidifying agent.

Formula per Litre:

Bacteriological Agar	13.5g	Bacteriological peptone	10.0g
Dipotassium phosphate	2g	Eosin Y	0.4g
Lactose	5g	Methylene blue	0.065g
Sucrose	5g		

Preparation:

Suspend 36 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. Cool to 45-50 ºC, mix well, avoiding the formation of bubbles and dispense carefully into Petri Dishes. DO NOT OVEARHEAT

Instructions for use:

For the isolation of enteric pathogens from clinical samples. For clinical diagnosis, the type of sample is urine and feces.
- Inoculate a plate of EMB Agar and streak for isolation, allowing discrete colonies to develop.
- Incubate at 35±2 °C and observe at 24 hours and again at 48 hours.

Colonies characteristics:
- Salmonella and Shigella colonies are translucent and amber colored or colorless.
- Coliforms that use lactose and/or sucrose produce blue-black colonies with dark centers and a greenish metallic sheen.
- Other coliforms such as Enterobacter form mucoid, pink colonies.
- Strains of Enterococcus faecalis are partially inhibited on this medium and appear as colorless colonies.

Note: As the medium is moderately inhibitory some staphylococci, streptococci and yeast may grow. Also some Gram-negative non-fermenting bacilli may appear as non-lactose fermenters. Further Biochemical tests are necessary for genus identification.

Quality control:

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Purple-rose flocculent precipitate	Tournasol blue. After sterilization: Orange	7.2 ± 0.2

Microbiological test:

Inoculation conditions: (35±2ºC / 24-48 h).

Microorganisms	Specification	Characteristic reaction
Pseudomonas aeruginosa ATCC 10145	Good growth	Colorless colony
Enterobacter aerogenes ATCC 13048	Good growth	Pink colony
Salmonella typhimurium ATCC 14028	Good growth	Colorless colony
Escherichia coli ATCC 25922	Good growth	Green colony with metallic shine
Staphylococcus aureus ATCC 25923	Inhibited growth	

Storage:

Once opened keep powdered medium closed to avoid hydration.

Bibliography:

American Public Heath Association. Diagnostic Procedures and Reagents. 2nd Ed. APHA, Inc. New York, 1950
A.P.H.A Examination of dairy products. 10th Ed. APHA, Inc. New York, 1953. Society of American Bacteriologists.
Manual of Microbiological Methods MacGraw-Hill New York, 1957.