EMB Agar

Intended Use

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

Typical Composition (g/litre)

Peptone 10.0; Dipotassium hydrogen phosphate 2.0; Lactose 5.0; Sucrose 5.0; Eosin-Y 0.400; Methylene blue 0.065; Agar 13.500

Mode of Action

The above medium is a combination of the Levine and Holt-Harris and Teague formula. 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. Nonfermenters probably raise the pH of surrounding medium by oxidative deamination of protein, which solubilizes the methylene blue-eosin complex resulting in colourless colonies. 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.

Preparation

Suspend 35.96 grams in 1 litre distilled water. 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 and to suspend the flocculent precipitate. Store the medium away from light to avoid photooxidation. Mix well before pouring in sterile Petri plates.

Final pH 7.2±0.2 (at 25°C)

Storage

Store between 10 - 30°C in tightly closed container and the prepared medium at 23-30°C. Use before expiry. date on the label.

Experimental Procedure and Evaluation

Depend on the purpose for which the media are used.

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.

Quality Control

Organism	Inoculum	Growth	Recovery
Escherichia coli ATCC 25922	50 - 100	Luxuriant	≥ 50 %
Staphylococcus aureus ATCC 25923	≥ 10^3	Inhibited	0 %
Klebsiella aerogenes ATCC 13048	50 - 100	Good	40-50%
Klebsiella pneumonia ATCC 13883	50 - 100	Good	40-50%
Proteus mirabilis ATCC 25933	50 - 100	Luxuriant	≥ 50 %
Salmonella typhimurium ATCC 14028	50 - 100	Luxuriant	≥ 50%

Reference

1. Holt-Harris and Teague,1916, J. Infect. Dis., 18 : 596.
2. Levine, 1918, J. Infect. Dis., 23:43.
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. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.