EMB Agar Base

M301

EMB Agar Base is a basal medium to which different carbohydrates and other test substances may be added for differentiation and study of various enteric bacteria.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Dipotassium phosphate	2.000
Eosin - Y	0.400
Methylene blue	0.065
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.46 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Add desired carbohydrate or other test substance in desired concentration . Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.DO NOT OVERHEAT. Cool to 45-50°C and shake the medium in order to oxidize the methylene blue (i.e. restore its blue colour) and to suspend the precipitate, which is an essential part of the medium. Mix well and pour into sterile Petri plates.

Precaution : Store the medium away from light to avoid photooxidation .

Principle And Interpretation

Levine EMB Agar was developed by Levine (1,2) and is used for the differentiation of *Escherichia coli* and *Enterobacter aerogenes* and also for the rapid identification of *Candida albicans* . This medium is recommended for the detection, enumeration and differentiation of members of the coliform group by American Public Health Association (3, 4, 5). Some gram-positive bacteria such as faecal Streptococci, yeasts grow on this medium and form pinpoint colonies. EMB Agar Base is a modification of EMB Agar, Levine without lactose. This facilitates the use of the medium as a basal agar to which desired carbohydrates could be added to differentiate between various enteric bacteria.

Eosin-Y and methylene blue make the medium slightly selective and inhibit certain gram-positive bacteria. These dyes differentiate between lactose fermenters and nonfermenters. The ratio of eosin-methylene blue is adjusted to approximately 6:1. Coliforms produce purplish black colonies due to uptake of methylene blue-eosin dye complex, when the pH drops. The dye complex is absorbed into the colony. Non-fermenters probably raise the pH of surrounding medium by oxidative de-amination of protein, which solubilizes the methylene blue-eosin complex resulting in formation of colourless colonies (6).

Peptone serves as source of carbon, nitrogen, and other essential growth nutrients. 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.

Quality Control

Appearance

Light pink to purple homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

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 2.75% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
Cultural characteristics observed with added carbohydrate after an incubation at 35-37°C for 18-24 hours (Fungal cultures incubated at 25-30°C for 24-48 hours).

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cultural Response				
Candida albicans ATCC 10231	50-100	luxuriant (incubated in 10% CO2)	>=50%	colourless
Escherichia coli ATCC 25922	50-100	luxuriant	>=50%	blue-black with green metallic sheen
Enterobacter aerogenes ATCC 13048	50-100	good	40-50%	pink-red
Enterococcus faecalis ATCC 29212	50-100	non-poor	<=10%	colourless
Pseudomonas aeruginosa ATCC 27853	50-100	luxuriant	>=50%	colourless
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	>=50%	colourless
Saccharomyces cerevisiae ATCC 9763	50-100	none-poor	<=10%	cream
Staphylococcus aureus ATCC 25923	50-100	none-poor	<=10%	colourless

Storage and Shelf Life
Store below 30°C in tightly closed container and prepared medium at 2 - 8°C and away from light. Use before expiry date on the label.

Reference
1. Levine M., 1918, J. Infect. Dis., 23:43.
2. Levine M., 1921, Bull. 62, Iowa State College Engr. Exp. Station.
3. Greenberg A. E., Trussell R. R. and Clesceri L. S. (Eds.), 1998, Standard Met for the Examination of Water and Wastewater, 20th ed., APHA, Washington, D.C.
4. Marshall R. (Ed.), 1992, Standard Methods for the Examination of Dairy ,, Products, 16th ed., APHA Inc., New York.
5. Downes F. P and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
6. Howard B. J., 1994, Clinical and Pathogenic Microbiology, 2nd Ed., Mosby Year Book, Inc

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