EMB Agar (Levine)

M022S

EMB Agar (Levine) is recommended for the isolation, enumeration or differentiation of members of *Enterobacteriaceae* .

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Dipotassium phosphate	2.000
Lactose	10.000
Eosin - Y	0.400
Methylene blue	0.065
Agar	15.000
Final pH (at 25°C)	7.1±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.5 grams in 1000 ml 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 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.

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). It is also recommended by BIS for detection and estimation of coliform bacteria in food stuff (6) and *Escherichia coli* from food and water (7). Eosin-Y and methylene blue make the medium slightly selective and inhibit certain gram-positive bacteria. These dyes differentiate between lactose fermenters and nonfermenters. Some gram-positive bacteria such as faecal Streptococci, yeasts grow on this medium and form pinpoint colonies. Weld (8, 9) proposed the use of Levine EMB Agar, with added Chlortetracycline hydrochloride, for the rapid identification of *Candida albicans* in clinical specimens. A positive identification of *Candida albicans* can be made after 24 - 48 hours incubation at 35 - 37°C in 10% carbon dioxide atmosphere, from specimens such as faeces, oral and vaginal secretions and nail or skin scraping etc. However, the typical appearance is variable.

Quality Control

Appearance
Light pink to purple coloured homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Reddish purple coloured slightly opalescent gel with greenish cast and finely dispersed precipitate, forms in Petri plates.

Reaction
Reaction of 3.75% w/v aqueous solution at 25°C. pH : 7.1±0.1

Cultural Response
M022S: Cultural characteristics observed after an incubation at 35 - 37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony

Cultural Response

Candida albicans ATCC 10231	50-100	good-luxuriant (Incubated in 10% carbon dioxide)	>=50%	colourless
Enterobacter aerogenes ATCC 13048	50-100	good	>=50%	pink-red
Escherichia coli ATCC 25922	50-100	luxuriant	>=50%	blue-black with metallic sheen
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0%	
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.), 1985, Standard Methods for the Examination of Water and Waste water, 16th 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. Speck M. (Ed.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd ed., APHA, Washington, D.C.
6. Bureau of Indian Standards, IS : 5401, 1969 (Second reprint - June 1990).
7. Bureau of Indian Standards, IS : 5887 (Part - I) 1976, reaffirmed 1986.
8. Weld J. T., 1952, Arch. Dermat. Syph., 66:691.
9. Weld J. T., 1953, Arch. Dermat. Syph., 67(5):433.

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