EMB Agar (Levine)

M022S

Intended Use

Recommended for isolation, enumeration and differentiation of members of *Enterobacteriaceae.* It is recommended by BIS committee under the specifications IS:5887 (Part I)-1976,Reaffirmed 2005, IS:5401(Part 1)-2012.

Composition**

Ingredients	g / L
Peptone	10.000
Dipotassium hydrogen 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 purified/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 (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 by BIS committee for isolation, identification and enumeration of*Escherichia coli* from foods (3) and estimation of coliform bacteria in food and animal feeding stuffs (4). It is also recommended for the detection, enumeration and differentiation of members of the coliform group by American Public Health Association (3,5,6).

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 (7,8) 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.

Type of specimen

Foodstuffs

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. A non-selective medium should be inoculated in conjunction with EMB Agar.
2. Confirmatory tests should be further carried out for identification of isolated colonies.
3. Some strains of *Salmonella* and *Shigella* species do not grow in the presence of eosin and methylene blue.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant (Incubated in 10% carbon dioxide)	>=50%	colourless
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	good	>=50%	pink-red
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=50%	blue-black with metallic sheen
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=50%	colourless
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	>=50%	colourless
Saccharomyces cerevisiae ATCC 9763 (00058*)	50-100	none-poor	<=10%	cream
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	none-poor	<=10%	colourless

Key: (*) Corresponding WDCM numbers (#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in 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.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

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2.Levine M., 1921, Bull. 62, Iowa State College Engr. Exp. Station.
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4.Bureau of Indian Standards, IS : 5887 (Part - I) 1976, reaffirmed 2005.
5.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.
6.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
7. Weld J. T., 1952, Arch. Dermat. Syph., 66:691.
8. Weld J. T., 1953, Arch. Dermat. Syph., 67(5):433.
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
10.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.

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