EMB Agar, Levine

M022

Intended Use:

EMB Agar (Levine) is recommended for the isolation, enumeration and differentiation of members of *Enterobacteriaceae* from clinical and non clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone	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±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.46 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 (7,8) and is used for the differentiation of *Escherichia coli* and *Klebsiella* 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 (2,9,10). Weld (11,12) 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.

Eosin Y and methylene blue make the medium slightly selective and inhibit certain gram-positive bacteria. These dyes serve as differential indicators in response to the fermentation of carbohydrates. This helps to differentiate between lactose-fermenters and non-fermenters in EMB Agar, Levine. 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 (4). Peptone serves as source of carbon, nitrogen, long chain amino acids, vitamins and other essential growth nutrients. Lactose serves as the source of energy by being the fermentable carbohydrate. 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.

Type of specimen

Clinical samples - urine , Foodstuffs; Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,3,10).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)

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

Warning and Precautions

In Vitro diagnostic use only. 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 clinical 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 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 3.75% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

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 (00054*)	50-100	luxuriant (incubated in 10% carbon dioxide)	>=50%	colourless
# Enterobacter aerogenes ATCC 13048 (00175*)	50-100	good	40-50%	pink-red
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=50%	blue-black with metallic sheen
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	<=10%	colourless
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=50%	colourless
Staphylococcus aureus ATCC 6538 (00032*)	50-100	none-poor	<=10%	colourless
Pseudomonas aeruginosa ATCC 9027 (00026*)	50-100	luxuriant	>=50%	colourless
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	>=50%	colourless
Saccharomyces cerevisiae ATCC 9763	50-100	none-poor	<=10%	cream
Staphylococcus aureus ATCC 25923 (00058*)	50-100	none-poor	<=10%	colourless
Escherichia coli NCTC 9002	50-100	luxuriant	>=50%	blue-black with green metallic sheen

Escherichia coli ATCC 8739 (00012*)	50-100	luxuriant	>=50%	blue-black with green metallic sheen

 # Formerly known as Enterobacter aerogenes

Storage and Shelf Life

Store below 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. Use before expiry date on the label. 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 (5,6).

Reference

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10. 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.
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IVD — In vitro diagnostic medical device

CE Marking

30°C / 10°C — Storage temperature

Do not use if package is damaged

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