Technical Data

EMB Agar, Levine Plate

MP022

Intended Use:

Recommended for the isolation, enumeration and differentiation of members of *Enterobacteriaceae* from clinical and non clinical samples.

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±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Principle And Interpretation

Levine EMB Agar was developed by Levine (1,2) 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 (3,4,5). 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. 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, faeces, oral and vaginal secretions and nail or skin scraping , Foodstuffs; Water samples.

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional 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. Some strains of *Salmonella* and *Shigella* species do not grow in the presence of eosin and methylene blue.
3. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
4. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
5. Confirmatory tests should be further carried out for identification of isolated colonies.
6. It is recommended to store the plates at 24-30°C to avoid minimum condensation.

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

Sterile EMB Agar, Levine in 90mm disposable plates with smooth surface and absence of black particles/cracks/bubbles

Colour

Reddish purple coloured medium with greenish cast.

Quantity of medium

25ml of medium in disposable plate

pH

6.90- 7.30

Sterility Check

Passes release criteria

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	luxuriant (incubated in 10% CO_2)	>=50%	colourless
# *Klebsiella 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
Escherichia coli ATCC 8739 (00012*)	50-100	Luxuriant	>=50%	Blue-black with metallic sheen
Pseudomonas aeruginosa ATCC 27853 (00025*)	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 subsp. *aureus* ATCC 25923 (00034*)	50-100	None-poor	<=10%	Colourless
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50-100	None-poor	<=10%	Colourless

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

Storage and Shelf Life

On receipt store between 20-30°C. 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 (8,9).

Reference

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4. Marshall R. (Ed.), 1992, Standard Methods for the Examination of Dairy , Products, 16th ed., APHA Inc., New York.
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6. Weld J. T., 1952, Arch. Dermat. Syph., 66:691.
7. Weld J. T., 1953, Arch. Dermat. Syph., 67(5):433.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. 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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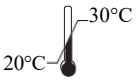
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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CE Marking

Do not use if package is damaged