

TM 1171 -EMB AGAR, LEVINE (IS : 5887 (Part I) 1976, reaffirmed 2005)

INTENDED USE

For isolation, enumeration and differentiation of *Enterobacteriaceae*.

PRODUCT SUMMARY AND EXPLANATION

EMB agar, Levine is used for the differentiation of *Escherichia coli* and *Klebsiella aerogenes* and also for the rapid identification of *Candida albicans*. It was developed by Levine. This medium is recommended for the detection, enumeration and differentiation of members of the coliform group by American Public Health Association. Weld proposed the use of Levine EMB Agar, with added Chlorotetracycline hydrochloride, for the rapid identification of *Candida albicans* in clinical specimens. It is also recommended by BIS for detection and estimation of coliform bacteria in food stuff and *Escherichia coli* from food and water.

COMPOSITION

Ingredients	Gms / Ltr
Agar	15.000
Peptic digest of animal tissue	10.000
Lactose	10.000
Dipotassium phosphate	2.000
Eosin – Y	0.400
Methylene blue	0.065

PRINCIPLE

This medium contains Eosin Y and methylene blue which makes 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. Coliform produces purplish black colonies due to uptake of methylene blue-eosin dye complex, when the pH drops. 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 colorless colonies. Lactose serves as the source of energy by being the fermentable carbohydrate. Peptic digest of animal tissue serves as source of carbon, nitrogen, and other essential growth nutrients. Eosin-Y and methylene blue serve as differential indicators. Phosphate buffers the medium.

INSTRUCTION FOR USE

- Dissolve 37.46 grams in 1000ml distilled water.
- Gently heat to boiling with gentle swirling and dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 45-50°C and mix well 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.

QUALITY CONTROL SPECIFICATIONS

- Appearance of Dehydrated powder** : Light pink to purple colour, homogeneous free flowing powder
- Appearance of Prepared medium** : Reddish purple coloured, opalescent gel with greenish cast and finely dispersed precipitate

pH (at 25°C) : 7.1± 0.1

INTERPRETATION

Culture Characteristics observed after incubation. Recovery is considered 100% on Soya Agar.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony	Incubation temperature	Incubation period
<i>Candida albicans</i>	10231	50-100	Luxuriant	>=50%	Colourless (Incubated in 10% carbon dioxide)	35-37°C	24-48 hours
<i>Escherichia coli</i>	25922	50-100	Luxuriant	>=50%	Black with green metallic sheen	35-37°C	24-48 hours
<i>Enterococcus faecalis</i>	29212	50-100	None-poor	<=10%	Colourless	35-37°C	24-48 hours
<i>Klebsiella aerogenes</i>	13048	50-100	Good	40-50%	Pink-red	35-37°C	24-48 hours
<i>Pseudomonas aeruginosa</i>	27853	50-100	Luxuriant	>=50%	Colourless	35-37°C	24-48 hours
<i>Staphylococcus aureus</i>	25923	50-100	None-poor	<=10%	Colourless	35-37°C	24-48 hours
<i>Salmonella Typhimurium</i>	14028	50-100	Luxuriant	>=50%	Colourless	35-37°C	24-48 hours
<i>Saccharomyces cerevisiae</i>	9763	50-100	None-poor	<=10%	Cream	35-37°C	24-48 hours

PACKAGING

In 100 & 500 gm packaging size.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct Sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use powder if they show evidence of microbial contamination, discoloration, drying, or other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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4. Greenberg A. E., Trussell R. R. and Clesceri L. S. (Eds.), 1998, Standard Methods ,, for the Examination of Water and Wastewater, 20th ed., APHA, Washington, D.C.
5. Bureau of Indian Standards, IS : 5401, 1969 (Second reprint - June 1990).
6. Bureau of Indian Standards, IS : 5887 (Part - I) 1976, reaffirmed 1986.



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8. Weld J. T., 1953, Arch. Dermat. Syph., 67(5):433.
9. Downes F. P and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
10. Howard B. J., 1994, Clinical and Pathogenic Microbiology, 2nd Ed., Mosby Year Book, Inc.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
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