# CM 20643-EMB AGAR, LEVINE (IS : 5887 (Part I) 1976, reaffirmed 2005)

## INTENDED USE

For isolation, enumeration and differentiation of Enterobacteriaceae.

## PRODUCT SUMMARY AND EXPLANATION

EMBagar,Levine isused forthe 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 Chlortetracycline 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

Thismediumcontains EosinYandmethylene blue which makes the medium slightly selective and inhibit certain gram-positive bacteria. These dyesserveasdifferential 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 approximately6:1.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 solubilizesthemethylene blue-eosin complex resulting in formation of colorless colonies. Lactose serves as the source of energy by beingthefermentable carbohydrate. Peptic digest of animal tissue serves as source of carbon, nitrogen, and otheressentialgrowthnutrients. Eosin-Y and methylene blue serve as differential indicators. Phosphate buffers the medium.

## INSTRUCTION FOR USE

Dissolve 37.46grams in 1000ml distilled water.
Gently heat toboiling with gentle swirling and dissolve the medium completely.
Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
Cool to 45-50°Cand 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 themedium 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 |
|---|---|---|---|---|---|---|---|
| Candida albicans | 10231 | 50-100 | Luxuriant | >=50% | Colourless (Incubated in 10% carbon dioxide) | 35-37°C | 24-48 hours |
| Escherichia coli | 25922 | 50-100 | Luxuriant | >=50% | Black with green metallic sheen | 35-37°C | 24-48 hours |
| Enterococcus faecalis | 29212 | 50-100 | None-poor | <=10% | Colourless | 35-37°C | 24-48 hours |
| Klebsiella aerogenes | 13048 | 50-100 | Good | 40-50% | Pink-red | 35-37°C | 24-48 hours |
| Pseudomonas aeruginosa | 27853 | 50-100 | Luxuriant | >=50% | Colourless | 35-37°C | 24-48 hours |
| Staphylococcus aureus | 25923 | 50-100 | None-poor | <=10% | Colourless | 35-37°C | 24-48 hours |
| Salmonella Typhimurium | 14028 | 50-100 | Luxuriant | >=50% | Colourless | 35-37°C | 24-48 hours |
| Saccharomyces cerevisiae | 9763 | 50-100 | None-poor | <=10% | Cream | 35-37°C | 24-48 hours |

## PACKAGING

In100&500gm packaging size.

## STORAGE

Dehydratedpowder, 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

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

## REFERENCES

1. Levine M., 1918, J. Infect. Dis., 23:43.
2. Levine M., 1921, Bull. 62, Iowa State College Engr. Exp. Station.
3. Marshall R. (Ed.), 1992, Standard Methods for the Examination of Dairy ,, Products, 16th ed., APHA Inc., New York.
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.

7. Weld J. T., 1952, Arch. Dermat. Syph., 66:691.
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