

# TM 371 – EMB AGAR, LEVINE

### **INTENDED USE**

For isolation, enumeration and differentiation of members of *Enterobacteriaceae* from pharma, dairy & food products.

### PRODUCT SUMMARY AND EXPLANATION

Levine EMB Agar was developed by Levine 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 for the detection, enumeration and differentiation of members of the coliform group by American Public Health Association. 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 |  |  |
|---------------------------------|-----------|--|--|
| Peptic digest of animal tissues | 10.00     |  |  |
| Dipotassium phosphate           | 2.000     |  |  |
| Lactose                         | 10.00     |  |  |
| Eosin -Y                        | 0.400     |  |  |
| Methylene blue                  | 0.065     |  |  |
| Agar                            | 15.000    |  |  |

## **PRINCIPLE**

Eosin-Y and methylene blue make the medium slightly selective and inhibit certain gram-positive bacteria. These dyes differentiate between lactose fermenters and non-fermenters. Some gram-positive bacteria such as faecal Streptococci, yeasts grow on this medium and form pinpoint colonies. Weld 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.

## **INSTRUCTION FOR USE**

- Dissolve the 37.5 grams in 1000 ml distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi 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 photo oxidation.

## **QUALITY CONTROL SPECIFICATIONS**













**Appearance of Powder** : Light pink to purple coloured homogeneous free flowing powder.

Appearance of prepared medium : Reddish purple coloured slightly opalescent gel with greenish cast and finely

dispersed precipitate, forms in Petri plates.

**pH (at 25°C)** :  $7.1 \pm 0.2$ 

## **INTERPRETATION**

Cultural characteristics observed after an incubation.

| Microorganism                         | ATCC  | Inoculum<br>(CFU/ml) | Growth                                                            | Recovery | Color of the colony                   | Incubation<br>Temperature | Incubation<br>Period |
|---------------------------------------|-------|----------------------|-------------------------------------------------------------------|----------|---------------------------------------|---------------------------|----------------------|
| Candida albicans                      | 10231 | 10-100               | Good-<br>Luxuriant<br>(Incubate<br>d in 10%<br>carbon<br>dioxide) | >=70%    | Colourless                            | 35-37°C                   | 24-48<br>Hours       |
| Enterobacter<br>aerogenes             | 13048 | 50-100               | Good                                                              | 40-50%   | Pink red                              | 35-37°C                   | 24-48<br>Hours       |
| Enterococcus<br>faecalis              | 29212 | >=10³                | Inhibited                                                         | 0%       | -                                     | 35-37°C                   | 24-48<br>Hours       |
| Escherichia coli                      | 25922 | 50-100               | Luxuriant                                                         | >=70%    | Blue- black<br>with metallic<br>sheen | 35-37°C                   | 24-48<br>Hours       |
| Pseudomonas<br>aeruginosa             | 27853 | 50-100               | Luxuriant                                                         | >=70%    | Colourless                            | 35-37°C                   | 24-48<br>Hours       |
| Saccharomyces<br>cerevisiae           | 9763  | 10-100               | None-<br>poor                                                     | 0-10%    | Cream                                 | 35-37°C                   | 24-48<br>Hours       |
| Salmonella<br>Serotype<br>Typhimurium | 14028 | 50-100               | Luxuriant                                                         | >=70%    | Colourless                            | 35-37°C                   | 24-48<br>Hours       |
| Staphylococcus<br>aureus              | 25923 | 50-100               | None-<br>poor                                                     | 0-10%    | Colourless                            | 35-37°C                   | 24-48<br>Hours       |

## **PACKAGING:**

In pack size of 100 gm and 500 gm bottles.













### **STORAGE**

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°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.

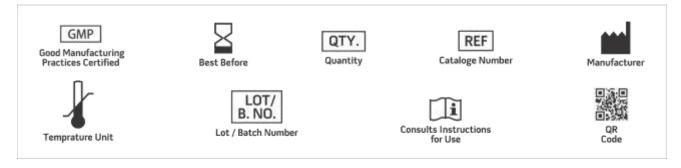
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

#### **DISPOSAL**

After use, 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. Greenberg A. E., Trussell R. R. and Clesceri L. S. (Eds.), 1985, Standard Methods for the Examination of Water and Waste water, 16th ed., APHA, Washington, D.C.
- 4. Marshall R. (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA Inc., New York.
- 5. Speck M. (Ed.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd ed., APHA, Washington, D.C.
- 6. Bureau of Indian Standards, IS: 5401, 1969 (Second reprint June 1990).
- 7. Bureau of Indian Standards, IS: 5887 (Part I) 1976, reaffirmed 1986.
- 8. Weld J. T., 1952, Arch. Dermat. Syph., 66:691.
- 9. Weld J. T., 1953, Arch. Dermat. Syph., 67(5):433.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices. \*For Lab Use Only

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