EMB Agar, Levine BIS

Intended Use
EMB Agar, Levine is recommended for isolation, enumeration and differentiation of members of *Enterobacteriaceae*. It is recommended by BIS committee under the specification IS:5887 (Part I)-1976, reaffirmed 2005, IS:5401 (Part 1)-2012.

Summary
Holt-Harris and Teague developed a culture medium for the differentiation of enteric microorganisms through the use of eosin and methylene blue dyes. Levine brought about a modification of their formulation, which he claimed gave a better differentiation between *Escherichia* and *Enterobacter* species. This Levine EMB Agar differs from the other formulation in not containing sucrose. The original medium could not discriminate between which carbohydrate (lactose or sucrose) was being fermented. Also, *Yersinia enterocolitica*, which ferments sucrose but not lactose, produced same colonies as that of lactose fermenters. Levine modified the formula by omitting sucrose and doubling the level of lactose. This medium was mainly developed to improve upon the differentiating properties of Endo Agar. EMB Agar, Levine is mainly used for the differentiation of *Escherichia coli* and *Enterobacter aerogenes* and also for the rapid identification of *Candida albicans*. It is recommended for use in microbial examination of dairy products, foods and water by APHA and for use in the performance of microbial limit test by USP and IP. It is also included in the Bacteriological Analytical Manual for food testing. Moreover, it is often the medicine of choice in many published reference methods, including the current Bureau of Indian Standards (BIS) method, for the isolation of *Enterobacteriaceae*. Weld proposed the use of EMB Agar, Levine with added chlortetracycline hydrochloride, for the rapid identification of *Candida albicans* in clinical specimens. A positive identification of *C. 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 scrapings. Menolasino et al., used this medium for identification of coagulase positive Staphylococci, which grew, as characteristic colourless pinpoint colonies.

Principle
Peptic digest of animal tissue provides essential nutrients while lactose is the fermentable carbohydrate. Dipotassium phosphate is the buffer. The Eosin Y and methylene blue dye in this medium inhibit Gram-positive organisms to a limited degree making the medium only slightly selective besides; it also helps in differentiating between lactose fermenters and non-lactose fermenters. Lactose fermenters produce blue-black colonies due to taking up of the dye when the pH drops. Lactose non-fermenters like Salmonella and Shigella probably raise the pH of the surrounding medium by oxidative deamination of the protein, solubilizing the methylene blue-eosin complex and appear as colourless or transparent colonies. Some Gram-positive bacteria like faecal *Streptococci, Staphylococci* and yeasts grow on this medium to form pinpoint colonies.

Formula*
Ingredients	**g/L**
Peptone	10.0
Lactose	10.0
Dipotassium Hydrogen Phosphate	2.0
Eosin Y	0.4
Methylene Blue	0.065
Agar	15.0
Final pH (at 25°C)	7.1 ± 0.1

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of specimen
Clinical samples - Faeces, Oral and Vaginal Secretions; Food and Dairy sample.

Specimen Collection and Handling

Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 37.46 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
5. AVOID OVER-HEATING.
6. Cool to 45°C-50°C and shake the medium in order to restore the blue colour (i.e. oxidize the methylene blue) and to suspend the precipitate, which is an essential part of the medium.
7. Mix well and pour into sterile petridishes.

Quality Control

Dehydrated Appearance: Light pink to purple coloured, homogeneous, free flowing powder.
Prepared Appearance: Deep red brown, slightly opalescent gel with or without fine precipitate, forms in petridishes.
Growth Promotion Test: Growth promotion is carried out in accordance with the method of BIS and growth is observed after incubation at 30°C-35°C for 18 to 24 hours.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism at 30°C-35°C for 18 hours.
Indicative Properties: The test results observed are within the specified temperature and time, inoculating ≤100 cfu of appropriate microorganism.

Growth Promoting + Indicative

Organism (ATCC)	Growth	Colour of Colony
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (14028)	Good	Colourless
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (23564)	Good	Colourless
Enterococcus faecalis (29212)	Partial Inhibition	Pinkish purple, pin point
Klebsiella pneumoniae subsp. *pneumoniae* (10031)	Good	Pink with blue centre, mucoid
Klebsiella aerogenes (13048)	Good	Pink with blue centre
Staphylococcus aureus subsp. *aureus* (25923)	Partial Inhibition	Colourless
Escherichia coli (25922)	Good	Purple with black centre and green metallic sheen
Proteus mirabilis (25933)	Good	Colourless
Candida albicans 3147 (10231)	Good	Colourless

Note: For good growth - Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. Store the medium away from light to avoid photo-oxidation
2. Some strains of *Salmonella* and *Shigella* will not grow in the presence of eosin Y and methylene blue.
3. Some Gram-positive bacteria such as Staphylococci, Enterococci and yeasts may grow on this medium.
4. Non-pathogenic, non-lactose fermenting organisms may also grow on this medium.
5. Serial inoculation may be required to assure adequate isolation of mixed flora samples.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Holt-Harris and Teague,1916. J. Iniec DB.18:596.
2. Levine, 1918.J. Intec.Dis..23:43.
3. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201050090100	Dehydrated Culture Media	100 g
201050090500	Dehydrated Culture Media	500 g