



EOSIN METHYLENE BLUE AGAR (LEVINE)

- For in vitro use only -

Catalogue No. PE60

Our Eosin Methylene Blue Agar (Levine) is a selective and differential medium used in the isolation of gram-negative enteric organisms from a variety of samples.

The Levine formulation of EMB Agar is a slight modification of Holt-Harris and Teague's original recipe from 1916. Unlike the Holt-Harris and Teague formulation, which contains two carbohydrate sources, the Levine formulation contains only one, lactose. This is beneficial since lactose fermenters can be differentiated from non-lactose fermenters. Pancreatic digest of gelatin provides a source of carbon, nitrogen, and other essential growth factors. The dyes, eosin Y and methylene blue, act both as differential indicators and inhibitors in the medium; the uptake of dyes during the growth cycle by some bacteria allows for differentiation between lactose fermenters and non-fermenters. Eosin Y is inhibitory to most gram-positive organisms although only to a limited degree; therefore some streptococci, staphylococci and yeasts may grow and form pinpoint colonies on EMB.

EMB Levine Agar is recommended by the APHA for the microbiological examination of dairy products and foods; it can also be used in the performance of microbial limit tests as outlined by the USP.

Formula per Litre of Medium

Pancreatic Digest of Gelatin	10.0 g
Lactose.....	10.0 g
Dipotassium Phosphate.....	2.0 g
Eosin Y	0.4 g
Methylene Blue	0.065 g
Agar	15.0 g

pH 7.1 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature.
2. Using an inoculum from the specimen, streak the plate as to obtain isolated colonies.
3. Incubate aerobically at 35°C.
4. Examine after 24 hours.
5. Incubate an additional 24 hours if no growth is observed.

Interpretation of Results

On EMB Agar (Levine), colony differentiation is due to the uptake of dyes by lactose fermenting organisms. The dyes, eosin and methylene blue, react to form a dark precipitate in an acid environment, therefore lactose fermenters take up the dyes giving colonies their typically blue-black coloration. Additionally, some rapid lactose fermenters, such as *E. coli*, form a surface, green metallic sheen due to the aldehydes formed during lactose fermentation. Non-lactose fermenters, such as *Salmonella* and *Shigella*, do not take up the dyes and appear as colorless or transparent colonies.

Additional biochemical and/or serological tests should be performed on isolated colonies from pure culture in order to complete identification.

- *The green metallic sheen associated with E. coli colonies may not always be present and is not diagnostic for E. coli*
- *Certain strains of Salmonella and Shigella are inhibited on EMB and may not grow, therefore a different selective, differential medium should be concurrently inoculated*

- *EMB is only moderately inhibitory and many gram-positive bacteria and yeast may grow on EMB, especially after prolonged incubation*
- *EMB medium is very sensitive to light and overexposure may lead to atypical colonial morphology and media deterioration*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
<i>Escherichia coli</i> ATCC 25922	Growth, blue-black colonies with metallic green sheen
<i>Salmonella typhimurium</i> ATCC 12022	Growth, colorless colonies
<i>Enterococcus faecalis</i> ATCC 29212	Partial inhibition

Storage and Shelf Life

Our EMB Agar (Levine) should be stored away from direct light at 4°C to 8°C. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 8 weeks from the date of manufacture.

References

1. Holt-Harris JE, Teague O. A new culture medium for the isolation of *Bacillus typhosa* from stools. J Infect Dis 1916; 18:596.
2. Levine MM. Differentiation of *E. coli* and *B. aerogenes* on a simplified eosin-methylene blue agar. J Infect Dis 1918; 23:43.
3. Girolami RL, Stamm JM. Inhibitory effect of light on growth-supporting properties of

- eosin methylene blue agar. Appl Environ Microbiol 1976; 31:141.
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 5. Difco Manual. 11th edition. Difco Laboratories: Maryland 1998.
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 7. Murray PR, Baron E, Pfaller M, Tenover F, Tenover R. Manual of Clinical Microbiology. 7th ed. Washington: ASM, 1999.
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 9. United States Pharmacopeial Convention, Inc. United States Pharmacopeia 25/National formulary 20 –2002. Rockville, MD: United States Pharmacopeial Convention, 2001.

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