

EOSIN METHYLENE BLUE AGAR (7134) (Holt, Harris & Teague)

Intended Use

Eosin Methylene Blue Agar (Holt, Harris & Teague) is used for the isolation and differentiation of Gram-negative enteric bacilli.

Product Summary and Explanation

Eosin Methylene Blue Agar (EMB) was developed by Holt-Harris and Teague.¹ This formula contains lactose and sucrose with two indicator dyes, Eosin Y and Methylene Blue. The use of Eosin Y and Methylene Blue as indicators produced sharp and distinct differentiation between colonies of lactose fermenting and nonfermenting organisms. Sucrose is included to detect coliforms that ferment sucrose more readily than lactose. EMB Agar is selective due to the presence of an inhibitor and differential based on the ability of some organisms to ferment carbohydrates with the absorption of an Eosin Y and Methylene Blue complex.

EMB Agar is recommended for use in examining clinical specimens for enteric pathogens.^{2,3}

Principles of the Procedure

Enzymatic Digest of Gelatin is the nitrogen source in EMB Agar. Lactose and Sucrose are the fermentable carbohydrates. Dipotassium Phosphate is the buffer. Eosin Y and Methylene Blue are dyes that combine to form a complex at an acid pH. At a sufficiently low pH, strong lactose fermenters such as *Escherichia coli* produce colonies with a green metallic sheen. These dyes act as both a pH indicator and an inhibitor. Gram-positive bacteria are partially inhibited on the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin.....	10 g
Lactose	5 g
Sucrose.....	5 g
Dipotassium Phosphate.....	2 g
Eosin Y.....	0.4 g
Methylene Blue	0.065 g
Agar	13.5 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, skin, and respiratory system.

Directions

1. Suspend 36 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light grey red-purple.

Prepared Appearance: Prepared medium is medium to dark red to blue-purple, with or without a fine precipitate, and trace to slightly hazy.

Expected Cultural Response: Cultural response on EMB Agar incubated at 35 ± 2°C and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
<i>Enterobacter aerogenes</i> ATCC® 13048	10 - 300	Growth	Blue-black bullseye
<i>Enterococcus faecalis</i> ATCC® 29212	1000	Partial inhibition	---
<i>Escherichia coli</i> ATCC® 25922	10 - 300	Growth	Blue-black bullseye; may have green metallic sheen
<i>Pseudomonas aeruginosa</i> ATCC® 27853	10 - 300	Growth	Colorless
<i>Salmonella typhimurium</i> ATCC® 14028	10 - 300	Growth	Colorless

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate fecal specimens and rectal swabs onto a small area of one quadrant of EMB Agar. Streak for isolation to permit development of discrete colonies. Examine plates at 24 and 48 hours for colonies with characteristic morphologies associated with potential pathogens.

Results

Colonies of *Salmonella* spp. and *Shigella* spp. are translucent, amber colored or colorless. Coliforms that use lactose and/or sucrose produce blue-black colonies with dark centers and a green metallic sheen. Other coliforms such as *Enterobacter* spp. form mucoid, pink colonies. Strains of *Enterococcus faecalis* are partially inhibited on this medium and are colorless pinpoint or very small colonies.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container.

Limitations of the Procedure

1. Some strains of *Salmonella* and *Shigella* may not grow on EMB Agar.⁴
2. EMB Agar is moderately inhibitory. Some staphylococci, streptococci and yeast may grow; they will appear as small, pinpoint colonies.
3. Sterilization reduces Methylene Blue, leaving the medium orange in color. The normal blue-purple color of the medium may be restored by gently mixing.
4. Not all strains of *E. coli* produce a green metallic sheen. The presence of the green metallic sheen is not diagnostic for *E. coli*.⁴

Packaging

Eosin Methylene Blue Agar (Holt, Harris & Teague)	Code No.	7134A	500 g
		7134B	2 kg
		7134C	10 kg

References

1. **Holt-Harris, J. E., and O. Teague.** 1916. A new culture medium for the isolation of *Bacillus typhosa* from stools. J. Infect. Dis. **18**:596.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Pezzlo, M.** 1992. Aerobic bacteriology, p. 1.0.1 – 1.20.47. In H. D. Isenberg (ed.). Clinical microbiology procedures handbook, vol. 1., American Society for Microbiology, Washington, D.C.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.