Eosin Methylene Blue (EMB) Agar Levine (NCM0105)

Intended Use
Eosin Methylene Blue Agar Levine is used for the isolation and differentiation of Gram-negative enteric bacilli and is not intended for use in the diagnosis of disease or other conditions in humans.

Description
This medium was introduced in 1916 by Holt-Harris and Teague to differentiate *Escherichia* spp. and *Aerobacter* spp. It was modified by Levine in 1918 who removed sucrose from the formula and increased the lactose content. The distinctive metallic sheen produced by *E. coli* on this medium is due to acid production resulting in an amide bonding between the eosin and methylene blue, other coliforms do not produce enough acid to cause this reaction. Eosin inhibits most Gram-positive organisms. The prepared medium is sensitive to light.

Typical Formulation

Peptone	10.0 g/L
Lactose	10.0 g/L
Dipotassium Hydrogen Phosphate	2.0 g/L
Eosin Yellow	0.4 g/L
Methylene Blue	0.065 g/L
Agar	15.0 g/L

pH: 6.8 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution
Refer to SDS

Preparation
1. Suspend 37.5 grams of the medium and add to 1 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.
4. Cool to 45-50°C and agitate gently to ensure uniform distribution of the flocculant precipitate which is a feature of this medium before pouring into Petri dishes.
5. STORE IN THE DARK.

Test Procedure
Refer to appropriate references for specific procedures using Eosin Methylene Blue Agar Levine.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, and pinkish-purple.

Prepared Appearance: Prepared medium is clear to hazy, with a slight precipitate, and reddish-brown.

Expected Cultural Response: Cultural response (next page) on Eosin Methylene Blue Agar Levine incubated at 37 ± 1°C and examined for growth after 21 - 27 hours.

Effective Date: 9/5/2019
Revision: 1

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Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
Enterococcus faecalis ATCC® 29212	~1000	Growth, suppressed colony size	---
Escherichia coli ATCC® 25922	50-200	>70% recovery	Purple-blue with green metallic sheen
Escherichia coli ATCC® 8739	50-200	>70% recovery	Purple-blue with green metallic sheen
Escherichia coli ATCC® 11775	4 Quad streak	Growth	Purple-blue with green metallic sheen
Salmonella typhimurium ATCC® 14028	4 Quad streak	Growth	Translucent

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

Results

Colonies of lactose fermenters are blue-black with or without a green metallic sheen. *E. coli* colonies typically are dark centered and usually have a green metallic sheen. Colonies of non-lactose fermenting bacteria are colorless and translucent. Refer to appropriate references for specific results and biochemical reactions.

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 when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. American Public Health Association, American Water Works Association and Water Pollution Control Federation, (1975). 2017. Standard methods for the examination of water and wastewater, 23rd ed. American Public Health Association, Washington, D.C.
2. Girolami, R.L. and Stamm, J.M. (1976). Inhibitory effect of light on growth supporting properties of Eosin Methylene Blue Agar. Appl. Environ. Microbiol., 31:1 141-142.
3. Haesler, W. J. (ed) (2004). Standard Methods for the Examination of Dairy Products, 17th ed., Washington, D.C., American Public Health Association.
4. Levine, M. (1918). Differentiation of *E. coli* and *B. aerogenes* on a simplified Eosin-Methylene Blue agar. J. Infect. Dis., 23: 43-47.

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