EOSIN METHYLENE BLUE AGAR, LEVINE (7103)

Intended Use
Eosin Methylene Blue Agar, Levine is used for the isolation and differentiation of Gram-negative enteric bacilli.

Product Summary and Explanation
Eosin Methylene Blue Agar, abbreviated EMB, was developed by Holt-Harris and Teague.[1] This formula contains lactose and sucrose with two indicator dyes, Eosin Y and Methylene Blue. Levine modified the formula by removing sucrose and doubling the concentration of lactose.[2,3] Eosin Methylene Blue Agar, Levine is used for testing clinical materials, food, and dairy products.[4-8] This medium is primarily used for the detection and confirmation of coliforms.

Principles of the Procedure
Enzymatic Digest of Gelatin is the nitrogen source in EMB Agar, Levine. Lactose is the carbohydrate and Dipotassium Phosphate is the buffer. Eosin Y and Methylene Blue are the indicators. Methylene Blue is also a selective agent. During strong acidic conditions, the dyes impart a metallic sheen to certain lactose fermenters, such as *E. coli*.

Formula / Liter
Enzymatic Digest of Gelatin .....10 g
Lactose .....10 g
Dipotassium Phosphate ..... 2 g
Eosin Y .....0.4 g
Methylene Blue .....0.065 g
Agar .....15 g
Final pH: 7.1 ± 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 37.5 g of the medium in one liter of purified water.
3. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
4. Autoclave at 121°C for 15 minutes.

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

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

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

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
Enterococcus faecalis ATCC® 29212	1000	Partial inhibition	---
Escherichia coli ATCC® 25922	10 - 300	Growth	Blue-black bullseye; may have green metallic sheen
Pseudomonas aeruginosa ATCC® 27853	10 - 300	Growth	Colorless

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

Test Procedure
Refer to appropriate references for specific procedures using Eosin Methylene Blue Agar, Levine.[4-8]

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.[4-8]

Storage
Store the 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 by keeping container tightly closed.

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.

Packaging

Eosin Methylene Blue Agar, Levine	**Code No.**	**7103A**	**500 g**
		7103B	**2 kg**
		7103C	**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. **Levine, M.** 1918. Differentiation of *E. coli* and *A. aerogenes* on a simplified eosin-methylene blue agar. J. Infect. Dis. **23:**43-47.
3. **Levine, M.** 1921. Bacteria fermenting lactose, the significance in water analysis. Bull. 62. Iowa State College Eng. Exp. Sta., Ames, Iowa.
4. **Cunnif, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16th ed. AOAC International, Gaithersburg, MD.
5. **http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManual/BAM/default.htm**
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food., 3rd ed. American Public Health Association, Washington, D.C.
7. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
8. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information
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