

Eosin Methylene Blue Agar, Modified, Holt-Harris and Teague

Intended Use

Eosin Methylene Blue Agar, Modified (formula of Holt-Harris and Teague) is a slightly selective and differential medium for the isolation, cultivation and differentiation of gram-negative enteric bacilli from both clinical and nonclinical specimens.

Summary and Explanation

In 1904, Endo developed a culture medium for the isolation of typhoid bacilli from feces,¹ and this medium was widely used in the years immediately following its development. According to Holt-Harris and Teague,² the chief disadvantage of the Endo medium was that the red color of the coliform colonies diffused through the surrounding medium. When larger numbers of these colonies were present on the agar surface, the colorless colonies of the typhoid organisms and other lactose nonfermenters were masked and often overlooked. In 1916, these two scientists reported on the development of a new medium in which the dyes, eosin Y and methylene blue, were incorporated. Differentiation between lactose fermenters and lactose nonfermenters on this formulation was greatly improved since color diffusion into the agar was eliminated.

The original EMB Agar formulation of Holt-Harris and Teague was modified by Levine who described his medium in a 1918 publication.³ Levine simplified the original formula by using a single peptone as a base and supplementing it with dipotassium phosphate as a buffer and by deleting the sucrose and increasing the concentration of lactose. The concentration of methylene blue was later reduced because of increased purity of the dye. This provided the current ratio of eosin to methylene blue of approximately 6:1. Over the years, it is the Levine Eosin Methylene Blue formulation that has achieved dominant status.

Principles of the Procedure

Eosin Methylene Blue Agar, Modified, contains eosin Y and methylene blue dyes that inhibit gram-positive bacteria to a limited degree. The dyes also serve as differential indicators in response to the fermentation of lactose and/or sucrose by microorganisms. Coliforms produce blue-black colonies due to the taking up of an eosin-methylene blue dye complex by the bacterial cells when the pH drops. *Salmonella* and *Shigella* colonies are colorless or have a transparent amber color. *Escherichia coli* colonies may show a characteristic green metallic sheen due to the rapid fermentation of lactose.

Some gram-positive bacteria, such as fecal streptococci, staphylococci and yeasts, will grow on this medium and usually form pinpoint colonies. A number of non-pathogenic, lactose-nonfermenting gram-negative bacteria will grow on this medium and must be distinguished from the pathogenic bacterial strains by additional biochemical tests.

User Quality Control

Identity Specifications

BBL™ Eosin Methylene Blue Agar, Modified, Holt-Harris and Teague

Dehydrated Appearance:	Fine, homogeneous, may contain up to a large amount of minute to small dark red purple particles.
Solution:	3.6% solution, soluble in purified water upon boiling. Solution is medium to dark, green orange brown, hazy.
Prepared Appearance:	Medium to dark, green orange brown, hazy.
Reaction of 3.6% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

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Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Enterococcus faecalis</i>	29212	10 ⁴ -10 ⁵	Partial inhibition
<i>Escherichia coli</i>	25922	10 ³ -10 ⁴	Good
<i>Proteus vulgaris</i>	9484	10 ³ -10 ⁴	Good
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhi	19430	10 ³ -10 ⁴	Good
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 ³ -10 ⁴	Good
<i>Shigella flexneri</i>	12022	10 ³ -10 ⁴	Good

Formula

BBL™ Eosin Methylene Blue Agar, Modified, Holt-Harris and Teague

Approximate Formula* Per Liter	
Pancreatic Digest of Gelatin	10.0 g
Lactose	5.0 g
Sucrose	5.0 g
Dipotassium Phosphate	2.0 g
Eosin Y	0.4 g
Methylene Blue	65.0 mg
Agar	13.5 g

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend 36 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Cool to approximately 45°C. Agitate gently and pour into plates.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen. Incubate plates, protected from light, at $35 \pm 2^\circ\text{C}$ for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

Expected Results

Typical colonial morphology on EMB Agar, Modified is as follows:

<i>Escherichia coli</i>	Large, blue-black, green metallic sheen
<i>Enterobacter/Klebsiella</i>	Large, mucoid, blue-black
<i>Proteus</i>	Large, colorless
<i>Salmonella</i>	Large, colorless to amber
<i>Shigella</i>	Large, colorless to amber
<i>Pseudomonas</i>	Irregular, colorless
Gram-positive bacteria.....	No growth to slight growth

References

1. Endo. 1904. Zentralbl. Bakteriol., Abt. 1 Orig. 35:109.
2. Holt-Harris and Teague. 1916. J. Infect. Dis. 18:596.
3. Levine. 1918. J. Inf. Dis. 23:43.

Availability

BBL™ Eosin Methylene Blue Agar, Modified, Holt-Harris and Teague

AOAC

Cat. No. 211215 Dehydrated – 500 g

United States and Canada

Cat. No. 221354 Prepared Plates – Pkg. of 20*
221355 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254014 Prepared Plates – Pkg. of 20*
254073 Prepared Plates – Ctn. of 120*

BBL™ Eosin Methylene Blue Agar, Modified, Holt-Harris and Teague//Columbia CNA Agar with 5% Sheep Blood

Cat. No. 221941 Prepared **I Plate™** Dishes – Pkg. of 20*

*Store at 2-8°C.