

# Eosin Methylene Blue Agar, Levine

## EMB Agar, Levine, without Lactose

### Intended Use

Eosin Methylene Blue Agar, Levine is a slightly selective and differential plating medium for the isolation of gram-negative enteric bacteria. EMB Agar, Levine, without Lactose is provided for convenience in genetic studies of enteric bacilli.

### Summary and Explanation

Shortly following the publication by Holt-Harris and Teague of a paper describing a new culture medium for the differentiation of enteric microorganisms through the use of eosin and methylene blue dyes,<sup>1</sup> Levine described a modification of their formulation which he claimed gave better differentiation between what are now referred to as *Escherichia* and *Enterobacter* species.<sup>2</sup> The two formulations differ in that Levine EMB Agar

does not contain sucrose. Both of these formulations were developed to improve upon the differentiating properties of Endo Agar,<sup>3</sup> which was developed previously.

Levine EMB Agar has become the predominant enteric plating medium that utilizes dyes as selective agents. It is recommended for use in the microbiological examination of dairy products and foods by the American Public Health Association.<sup>4,5</sup> EMB Agar, Levine, without Lactose can be supplemented with a carbohydrate as the sole carbon source for the study of hybrid enteric bacilli.<sup>6</sup>

### Principles of the Procedure

The eosin Y and methylene blue dyes in Levine EMB Agar render the medium slightly selective in that they inhibit gram-User

### Quality Control

#### Identity Specifications

##### BBL™ Eosin Methylene Blue Agar, Levine

Dehydrated Appearance: Fine, homogeneous, may contain up to a large amount of minute to small dark red purple particles.

Solution: 3.74% 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 3.74% Solution at 25°C: pH 7.1 ± 0.2

##### BBL™ EMB Agar, Levine, without Lactose

Dehydrated Appearance: Fine, homogeneous, may contain up to a large amount of minute to small dark red purple particles.

Solution: 2.74% 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 2.74% Solution at 25°C: pH 7.1 ± 0.2

#### Cultural Response

##### BBL™ Eosin Methylene Blue Agar, Levine or EMB Agar, Levine, without Lactose

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 <sup>4</sup> -10 <sup>5</sup>	Partial inhibition
<i>Escherichia coli</i>	25922	10 <sup>3</sup> -10 <sup>4</sup>	Good
<i>Klebsiella pneumoniae</i>	33495	10 <sup>3</sup> -10 <sup>4</sup>	Good
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhi	19430	10 <sup>3</sup> -10 <sup>4</sup>	Good
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 <sup>3</sup> -10 <sup>4</sup>	Good
<i>Shigella dysenteriae</i>	9361	10 <sup>3</sup> -10 <sup>4</sup>	Good
<i>Shigella flexneri</i>	12022	10 <sup>3</sup> -10 <sup>4</sup>	Good

*Escherichia coli*  
ATCC™ 25922



positive bacteria to a limited degree. These dyes also play a role in differentiating between lactose fermenters and lactose nonfermenters due to the presence or absence of dye uptake in the bacterial colonies. Coliforms, as lactose-fermenting organisms, are visualized as blue-black colonies, whereas colonies of *Salmonella* and *Shigella*, as lactose nonfermenters, appear colorless, transparent or amber.

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

## Formulae

### BBL™ Eosin Methylene Blue Agar, Levine

Approximate Formula* Per Liter	
Pancreatic Digest of Gelatin .....	10.0 g
Lactose .....	10.0 g
Dipotassium Phosphate .....	2.0 g
Eosin Y.....	0.4 g
Methylene Blue.....	65.0 mg
Agar .....	15.0 g

### BBL™ EMB Agar, Levine, without Lactose

Consists of the same ingredients without the lactose.

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

## Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
  - BBL™ Eosin Methylene Blue Agar, Levine – 37.4 g;
  - BBL™ EMB Agar, Levine, without Lactose – 27.4 g.
 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. 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 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

Follow established procedures when using the medium without lactose.

## Expected Results

Typical colonial morphology on Eosin Methylene Blue Agar, Levine 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
<i>Shigella</i> .....	Large, colorless
<i>Pseudomonas</i> .....	Irregular, colorless
Gram-positive bacteria .....	No growth to slight growth

Results obtained with Levine EMB Agar without Lactose are dependent upon the substituted carbohydrate.

## References

1. Holt-Harris and Teague. 1916. J. Infect. Dis. 18:596.
2. Levine. 1918. J. Infect. Dis. 23:43.
3. Endo. 1904. Zentralbl. Bakteriol., Abt. 1, Orig. 35:109.
4. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
6. Baron, Spilman and Carey. 1959. Abstr. G7, p. 29. Bacteriol. Proc. 59th Gen. Meet. Soc. Am. Bacteriologists 1959.

## Availability

### BBL™ Eosin Methylene Blue Agar, Levine

AOAC BAM BS12 CCAM CMPH2 COMPF MCM9 SMD

Cat. No.	211221	Dehydrated – 500 g
	211222	Dehydrated – 5 lb (2.3 kg)
	221170	Prepared Plates – Pkg. of 20*
	221268	Prepared Plates – Ctn. of 100*

### BBL™ EMB, Levine, without Lactose

Cat. No.	211191	Dehydrated – 500 g
----------	--------	--------------------

### BBL™ Eosin Methylene Blue Agar, Levine// Columbia CNA Agar with 5% Sheep Blood

Cat. No.	295618	Prepared I Plate™ Dishes – Ctn. of 100*
----------	--------	---

### BBL™ Eosin Methylene Blue Agar, Levine// MacConkey II Agar

Cat. No.	295969	Prepared I Plate™ Dishes – Ctn. of 100*
----------	--------	---

### BBL™ Eosin Methylene Blue Agar, Levine// Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

Cat. No.	221286	Prepared I Plate™ Dishes – Pkg. of 20*
----------	--------	--

\*Store at 2-8°C.