



EMB Levine / TSA Blood

Bi-plate for the isolation, cultivation and differentiation of pathogenic enterobacteria from clinical and nonclinical specimens.

DESCRIPTION

EMB Levine / TSA Blood is a ready-to-use plate containing two distinct and selective media used for the detection of clinically significant enteric bacteria.

Eosin Methylene Blue Levine Agar complies with the recommendations of the APHA for the microbiological examination of dairy products and food.

Tryptic Soy Agar with Sheep Blood allows the growth of fastidious organisms and the determination of hemolytic reactions.

TYPICAL FORMULA

<u>Eosin Methylene Blue Levine Agar</u>	(g/l)	<u>Tryptic Soy Agar with 5% Sheep Blood</u>	(g/l)
Enzymatic Digest of Gelatin	10.0	Enzymatic Digest of Casein	15.0
Lactose	10.0	Enzymatic Digest of Soybean Meal	5.0
Dipotassium Phosphate	2.0	Sodium Chloride	5.0
Eosin Y	0.4	Sheep Blood Defibrinated	50.0 ml
Methylene Blue	0.065	Agar	15.0
Agar	15.0	Final pH 7.3 ± 0.2 at 25°C	
Final pH 7.1 ± 0.2 at 25°C			

METHOD PRINCIPLE

In EMB Levine Agar, enzymatic digest of gelatin provides amino acids, nitrogen, carbon, vitamins and minerals required for organisms growth. Lactose is the fermentable carbohydrate. Dipotassium phosphate acts as buffer. Eosin Y and methylene blue play the double role of selective and differentiating agents. They inhibit Gram-positive bacteria to a limited degree and allow to distinguish blue-black colonies of coliforms from lactose non-fermenting organisms, such as *Salmonella* and *Shigella* spp, which form colorless colonies. Agar is the solidifying agent.

In Tryptic Soy Agar with Sheep Blood, enzymatic digest of casein and enzymatic digest of soybean meal provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Sodium chloride maintains the osmotic balance of the medium. Defibrinated sheep blood supplies additional growth factors for fastidious organisms and allows to evidence hemolytic reactions. Agar is the solidifying agent.

TEST PROCEDURE

Inoculate the plate by direct streaking or spread method. Incubate at 35 ± 2°C for 18-24 hours in an aerobic or CO₂-enriched atmosphere.

RESULTS INTERPRETATION

After incubation examine for growth and interpret the results as indicated in the ID table.

ID Table.

Eosin Methylene Blue Levine Agar		Tryptic Soy Agar with 5% Sheep Blood	
Microorganism	Appearance of colonies	Microorganism	Appearance of colonies
<i>E. coli</i>	Large, blue-black, green metallic sheen	Hemolytic streptococci	Translucent or opaque, grayish, small or large and mucoid, with zones of hemolysis
<i>Enterobacter</i> and <i>Klebsiella</i> spp	Large, mucoid, blue-black	Pneumococci	Very flat, smooth, translucent, grayish with zones of α-hemolysis
<i>Proteus</i> , <i>Salmonella</i> and <i>Shigella</i> spp	Large, colorless	Staphylococci	Opaque, white to gold-yellow with or without zones of β-hemolysis
<i>Pseudomonas</i> spp	Irregular, colorless	<i>Listeria</i> spp	Small zones of β-hemolysis

Notice that some Gram-positive bacteria, such as fecal streptococci, staphylococci and yeasts can grow on EMB Levine Agar and form pinpoint colonies.

APPEARANCE

Eosin Methylene Blue Levine Agar: opalescent, green orange brown.

Tryptic Soy Agar with 5% Sheep Blood: slightly opalescent, cherry red.

STORAGE AND TRANSPORT CONDITIONS

2-8°C away from light, until the expiry date on the label. However, our stability studies have shown that the transport at 18-25°C for 4 days, or at 35-39°C for 48 hours, does not alter in any way the performance of the product. Eliminate if signs of deterioration or contamination are evident.

SHELF LIFE

2 months.

QUALITY CONTROL

Plates are inoculated with the microbial strains indicated in the QC table.

Inoculum for productivity: 50-100 CFU

Inoculum for selectivity: 10^4 - 10^6 CFU

Incubation conditions: $35 \pm 2^\circ\text{C}$ for 18-24 hours in aerobic atmosphere (supplemented with 5-10% CO_2 for streptococci).

QC Table.

Microorganism		Growth on EMB Levine Agar	Growth on TSA Blood Agar
<i>Escherichia coli</i>	ATCC® 25922	Good, blue colonies with metallic sheen	Good, slight β -hemolysis
<i>Enterobacter cloacae</i>	ATCC® 13047	Good, blue colonies	Good
<i>Salmonella</i> Typhimurium	ATCC® 14028	Good, colorless colonies	Good
<i>Staphylococcus aureus</i>	ATCC® 25923	Inhibited	Good, β -hemolysis
<i>Streptococcus pyogenes</i>	ATCC® 19615	Inhibited	Good, β -hemolysis
<i>Streptococcus pneumoniae</i>	ATCC® 6305	Inhibited	Good, α -hemolysis

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for *in vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulation in force.

BIBLIOGRAPHY

- Wehr, H.M. and J.H. Frank ed. (2004) Standard methods for the examination of dairy products, 17th ed. American Public Health Association. Washington, D.C.
- Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Tenover, and R.H. Tenover ed. (2003) Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
- Levine, M. (1918) Differentiation of *B. coli* and *B. aerogenes* on a simplified eosin-methylene blue agar. J. Inf. Dis. 23:43-47.

PRESENTATION	Packaging	Ref.
EMB Levine / TSA Blood	20 plates	18020
EMB Levine / TSA Blood	100 plates	18020*

TABLE OF SYMBOLS

LOT Batch code	IVD <i>In vitro</i> Diagnostic Medical Device	 Manufacturer	 Use by	 Fragile, handle with care
REF Catalogue number	 Temperature limitation	 Contains sufficient for <n> tests	 Caution, consult Instruction For Use	 Do not reuse



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