

Levine Agar (EMB) BAM/ISO

Cat. 1050

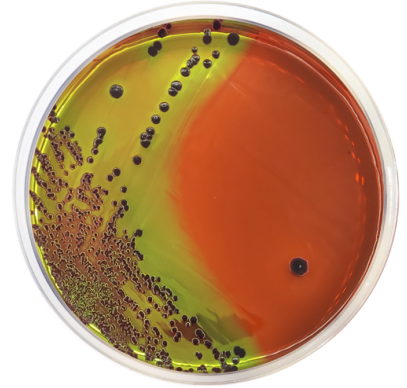
For the isolation and differentiation of Enterobacteria.

Practical information

Applications	Categories
Differentiation	Enterobacteria

Industry: Clinical / Food / Dairy products

Regulations: ISO 21150 / BAM



Principles and uses

Levine Agar (EMB) is a slightly selective medium for the investigation and differentiation of lactose-fermenting and lactose non-fermenting Enterobacteria in foods, dairy products and clinical samples. It is used for the examination of samples of sanitary importance for the presence of coliforms.

Levine Agar (EMB) is recommended by the normative ISO 21150 for the identification of Escherichia coli in comestic products.

Gelatin Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Eosin Y and Methylene blue are inhibitors of Gram positive bacteria. Bacteriological agar is the solidifying agent.

Coliforms, being lactose-fermenting organisms, are identified as blue-black colonies. Lactose non-fermenters, such as Salmonella and Shigella, show colorless colonies, transparent or amber.

Formula in g/L

Bacteriological agar	15	Dipotassium phosphate	2
Eosin Y	0,4	Gelatin peptone	10
Lactose	10	Methylene blue	0,065

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 37,5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45-50 °C, mix well and dispense into plates.

Instructions for use

» For clinical diagnosis, the type of sample is oral expectoration, vaginal secretions, skin and nasal scrapes.

- Inoculate over the surface in parallel striae with the inoculating loop or hyssop.
- Incubate in aerobic conditions at 35±2 °C for 18-24 hours.
- Reading and interpretation of the results.

» For other uses not covered by the CE marking:

For the confirmation of E. coli in food samples according to the BAM:

- Transfer a loopful of each gas positive LST tube (Cat. 1310) to an EC tube (Cat. 1522). A positive result in the EC broth is indicative of fecal coliforms presence.
- Inoculate each gas positive EC tube in an Levine Agar (EMB) agar plate and incubate at 35±0,5 °C for 18-24 hours.

- Suspicious *E. coli* colonies are dark centered and flat, with or without metallic sheen.
- Transfer up to 5 suspicious colonies from each Levine Agar (EMB) BAM/ISO (Cat. 1056) plate to PCA (Cat. 1056) slants, incubate them for 18-24 h at 35±0,5 °C and use for confirmation by biochemical tests.

For the identification of *Escherichia coli* in domestic products according to ISO 21150:

- Inoculate the surface of the Levine Agar (EMB) BAM/ISO (Cat.1050) medium with suspect isolated colonies grown on MacConkey Agar (Cat. 1052).
- Incubate at 32,5±2,5 °C for at least 24 h (maximum 48 h).
- The morphological characteristics of *Escherichia coli* are metallic sheen under reflected light and blue-black appearance under transmitted light.

Characteristics of the colonies:

- *Escherichia coli*: 2 - 3 mm in diameter. Blue-black in the center, with edges clear to transmitted light, often with a metallic green sheen with reflected light.
- *Salmonella* and *Shigella*: Transparent, amber to colorless.
- *Staphylococcus*: (coagulase-positive): Punctiform, colorless.
- *Enterobacter aerogenes*: Large, 4 - 6 mm in diameter. Elevated and mucoid. Grayish-brown in the center to transmitted light. Generally does not have a metallic sheen.
- *Proteus*: When there is no swarming, similar to *Salmonella* or *Shigella*.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Pink-reddish	Purple-blue	7,1±0,2

Microbiological test

Incubation conditions: (35±2 °C / 18-24 h)

Microorganisms	Specification	Characteristic reaction
<i>Klebsiella aerogenes</i> ATCC 13048	Good growth	Pink colony
<i>Salmonella typhimurium</i> ATCC 14028	Good growth	Colorless colony
<i>Escherichia coli</i> ATCC 25922	Good growth	Blue-black with green sheen, black center
<i>Staphylococcus aureus</i> ATCC 25923	Inhibited growth	
<i>Proteus mirabilis</i> ATCC 29906	Good growth	Colorless colony

Storage

Temp. Min.:2 °C
Temp. Max.:25 °C

Bibliography

BAM Media M80: Levine's Eosin-Methylene Blue (L-EMB) Agar

Levine, J. Inf. Dis. 22:43. 1981. J. Bact. 45:471. 1943. Vogel, R.A. and Moses, R.M. Weld's Method for the Rapid Identification of *Candida albicans* in Clinical Materials. Am. J. Clin. Path. 28:103-106. 1957.