

Eosin Methylene Blue Agar (EMB Agar)

Art. No. 01-068

Specification

Selective differential medium for the isolation and enumeration of coliforms according to ISO 21150 standard and USP.

Formula* in g/L

Peptone.....	10,000
Lactose.....	10,000
Dipotassium hydrogen phosphate.....	2,000
Eosin Y.....	0,400
Methylene blue.....	0,065
Agar.....	15,000
Final pH 6,90 ± 0,2 at 25°C	

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

Directions

Add 37,5 g to 1 L of distilled water. Bring to the boil and distribute in suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description

A very versatile medium originally developed for the differentiation of *E.coli* and *Enterobacter aerogenes*. It has also proved very effective in the rapid identification of *Candida albicans* and demonstrates a high correlation with the coagulase test for staphylococci.

It has been repeatedly recommended for the detection, enumeration and differentiation of members of the coliform group of bacteria.

Technique

The Weld method for the identification of *Candida albicans* uses this medium with chlortetracycline (100 mg/l) in a 10% CO₂ environment. The method's efficacy has been tested with a variety of samples, such as sputum, oral secretions, faeces, nails and vaginal secretions, all of which provide definitive results within 24-48 hours. Staphylococci are also easily identified, particularly coagulase-positive strains. These have a very characteristic appearance: small colourless colonies with a central red nucleus. The medium's prevailing application is in the differentiation of *E. coli* and *E. aerogenes*.

The medium should be sterilized once distributed into tubes containing 20 mL of product each, and then refrigerated. **Melt in a boiling water bath before use and stir until it acquires a dark purple colour.** Pour a tube into each sterile plate and allow it to solidify. It is advisable to dry the medium's surface before use, leaving the plate open but inverted.

For each doubtful lactose broth tube, inoculate one plate by streaking, and incubate for 24 to 48 hours at 37°C.

- *Escherichia coli* and *Citrobacter* form flat colonies of 2-3 mm in diameter and are dark violet in colour with a black centre which produces a distinctive green metallic sheen when light is reflected on it.
- *Enterobacter* and *Klebsiella* form convex colonies which are twice as big as the very smooth *E. coli*, have no metallic sheen and are pink in colour with a dark blue centre. Non-lactose fermenting organisms produce colourless colonies.
- *Candida albicans* colonies incubated in a CO₂ atmosphere have a very peculiar cotton-like appearance which distinguishes them from other *Candida species* that produce classical yeast like colonies.

References

- CLESCERI, L.S., A.E. GREENBERG & A.D. EATON (1998) Standard Methods for the Examination of Water and Wastewater. 20th ed. APHA-AWWA-WEF. Washington, DC.
- HOLT-HARRIS, J. E. y TEAGUE O.A. (1916) A New Culture Medium for the Isolation of *Bacillus typhosus* from Stools J. Infect. Dis. 18:596-600.
- ISO STANDARD 21150 (2006) Cosmetics. Microbiology - Detection of *Escherichia coli*.
- LEVINE, M (1918) Differentiation of *E. coli* and *A. aerogenes* on simplified Eosin-ethylene Blue Agar. J. Infect. Dis. 23:43-47.
- MENOLASINO, N.I., GRIEVES B. Y PAYNE P. (1960) Isolation and Identification of Coagulase Positive Staphylococci on Levine's Eosin-Methylene Blue Agar. J. Lab. Clin. Med. 56(6) 908-910.
- USP 31 - NF 26 (2008) <61> Microbial Tests. USP Con. Inc. Rockville, MD, USA.
- WELD, J. (1953) *Candida albicans*: Rapid Identification in Cultures made directly from Human materials Arch. Dermat. Syph. 67(5):473-478.
- WINDLE TAYLOR, E. (1958) The Examination of Water and Water Supplies. Churchill Ltd. 7th ed. Londres.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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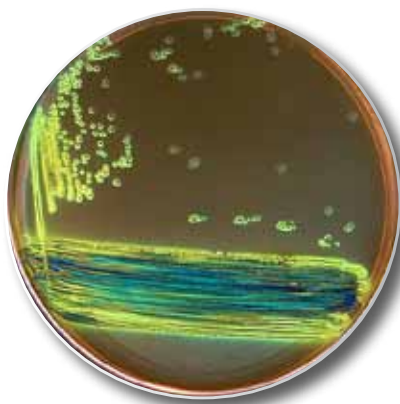
Quality control

Incubation temperature: 35°C ± 2.0

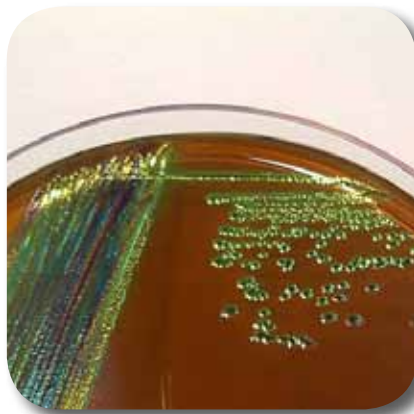
Incubation time: 24 h

Inoculum: Streak isolation

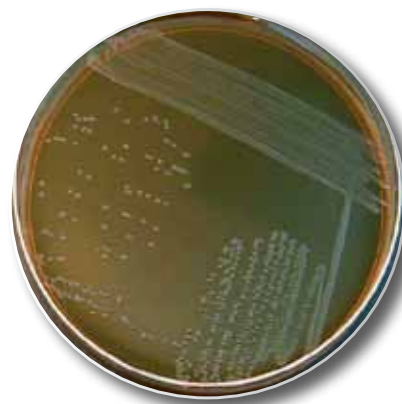
Microorganism	Growth	Remarks
<i>Enterococcus faecalis</i> ATCC 29212	Inhibited to poor	48 h
<i>Salmonella abony</i> NCTC 6017	Good to very good	Colourless colonies w/o green metallic shine
<i>Escherichia coli</i> ATCC 11775	Good to very good	Dark violet colonies w. green metallic sheen
<i>Escherichia coli</i> ATCC 25922	Good to very good	Dark violet colonies w. green metallic sheen
<i>Escherichia coli</i> ATCC 8739	Good to very good	Dark violet colonies w. green metallic sheen
<i>Salmonella typhimurium</i> ATCC 14028	Good to very good	Colourless colonies w/o green metallic shine
<i>Pseudomonas aeruginosa</i> ATCC 27853	Good to very good	Colourless colonies w/o green metallic sheen



Escherichia coli ATCC 8739



Escherichia coli ATCC 8739
"Detail"



Salmonella typhimurium ATCC 14028