Levine Eosin-Methylene Blue Agar Medium

MU022

Levine Eosin-Methylene Blue Agar Medium is recommended for the isolation, enumeration and differentiation of members of *Enterobacteriaceae* in accordance with United States Pharmacopoeia 2009.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of gelatin	10.000
Dibasic potassium phosphate	2.000
Lactose	10.000
Eosin - Y	0.400
Methylene blue	0.065
Agar	15.000
pH after sterilization (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.46 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. AVOID OVERHEATING. Cool to 50°C and shake the medium in order to oxidize the methylene blue (i.e. restore its blue colour) and to suspend the precipitate, which is an essential part of the medium. Mix well before pouring into sterile Petri plates.

Precaution: Store the medium away from light to avoid photooxidation.

Principle And Interpretation

Levine Eosin Methylene Blue Agar Medium was developed by Levine (1,2) and is used for the differentiation of Escherichia coli and *Enterobacter aerogenes* and also for the rapid identification of *Candida albicans* . This medium is recommended for the detection, enumeration and differentiation of members of the coliform group by American Public Health Association and United States Pharmacopoeia (3, 4, 5, 6).

Eosin-Y and methylene blue makes the medium slightly selective and inhibit certain gram-positive bacteria. Both dyes act as indicator and inhibiting agent. These dyes differentiate between lactose fermenters and non-fermenters. Eosin Y and methylene blue forms a complex at acidic pH, which acts as inhibiting agent. Essential nutrients and growth factors are provided by pancreatic digest of gelatin. Phosphates acts as good buffering agent. *E.coli* forms colonies with green metallic sheen, indicating strong lactose fermentation. Weld (7,8) proposed the use of Levine EMB Agar, with added Chlortetracycline hydrochloride, for the rapid identification of *Candida albicans* in clinical specimens. Some gram-positive bacteria such as faecal Streptococci, yeasts grow on this medium and form pinpoint colonies A positive identification of *Candida albicans* can be made after 24-48 hours incubation at 35-37°C in 10% carbon dioxide atmosphere, from specimens such as faeces, oral and vaginal secretions and nail or skin scraping etc. However, the typical appearance is variable.

Quality Control

Appearance

Light pink to purple homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Reddish purple with greenish cast coloured opalescent gel with finely dispersed precipitate forms in Petri plates.

pH

6.90-7.30

Cultural Response

Growth Promotion is carried out in accordance with USP. Cultural response was observed after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar and fungal growth on Sabouraud Dextrose Agar.

Cultural Response

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation temperature	Incubation period
Test for specified microorganism						
Escherichia coli ATCC 8739	50 -100	25 -100	>=50 %	blue-black colonies with metallic sheen	30 -35 °C	24 -48 hrs
Additional Microbiological testing						
Escherichia coli NCTC 9002	50 -100	25 -100	>=50 %	blue-black colonies with metallic sheen	30 -35 °C	24 -48 hrs
Escherichia coli ATCC 25922	50 -100	25 -100	>=50 %	blue-black colonies with metallic sheen	30 -35 °C	24 -48 hrs
Enterobacter aerogenes ATCC 13048	50 -100	25 -100	>=50 %	pink to red	30 -35 °C	24 -48 hrs
Salmonella Typhimurium ATCC 14028	50 -100	25 -100	>=50 %	colourless	30 -35 °C	24 -48 hrs
Pseudomonas aeruginosa ATCC 9027	50 -100	25 -100	>=50 %	colourless	30 -35 °C	24 -48 hrs
Enterococcus faecalis ATCC 29212	>=10^3	0	0 %		30 -35 °C	24 -48 hrs
Staphylococcus aureus ATCC 6538	>=10^3	0	0 %		30 -35 °C	24 -48 hrs
Candida albicans ATCC 10231	50 -100	25 -100	>=50 %	colourless	30 -35 °C	24 -48 hrs
Saccharomyces cerevisiae ATCC 9763	50 -100	0 -10	0 -10 %	cream	30 -35 °C	24 -48 hrs

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2 - 8°C and away from light. Use before expiry date on the label.

Reference

1. Levine M., 1918, J. Infect. Dis., 23:43.
2. Levine M., 1921, Bull. 62, Iowa State College Engr. Exp. Station.
3. Eaton A. D., Clesceri L. S. and Greenberg A W.,(Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington, D.C.
4. Wehr H M and Frank J H., 2004, Standard Methods for the Examination of Dairy Products, 17th ed., APHA Inc., Washington, D.C.
5. Downes F P and Ito K. (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
6. The United States Pharmacopoeia 2009, US Pharmacopoeial Convention Inc., Rockville, ,,M.D
7. Weld J. T., 1952, Arch. Dermat. Syph., 66:691.
8. Weld J. T., 1953, Arch. Dermat. Syph., 67(5):433.

Revision : 2 / 2015