EMB Agar, Levine (Levine Eosin-Methylene Blue Agar Medium)

MAP022

(MU022/ MM022)

Intended Use:

Recommended for the isolation, enumeration and differentiation of members of *Enterobacteriaceae* in accordance with USP/IP.

Composition**

Ingredients	g / L
Gelatine peptone #	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
Pancreatic digest of gelatin

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, United States Pharmacopoeia and Indian Pharmacopoeia (3-7).

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 (8,9) 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.

Type of specimen

Pharmaceutical, Water , Food samples.

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,5). For pharmaceutical products, follow appropriate techniques for sample processing in case of viscous materials as mentioned under sterility (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate
3. the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
4. A non-selective medium should be inoculated in conjunction with EMB Agar.
5. Confirmatory tests should be further carried out for identification of isolated colonies.
6. Some strains of *Salmonella* and *Shigella* species do not grow in the presence of eosin and methylene blue.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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/IP. 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 (00012*)	50 -100	25 -100	>=50 %	blue-black colonies with metallic sheen	30 -35 °C	24 -48 hrs
Additional Microbiological testing						
Escherichia coli ATCC 25922 (00013*)	50 -100	25 -100	>=50 %	blue-black colonies with metallic sheen	30 -35 °C	24 -48 hrs
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50 -100	25 -100	>=50 %	pink to red	30 -35 °C	24 -48 hrs
Salmonella Typhimurium ATCC 14028(00031*)	50 -100	25 -100	>=50 %	colourless	30 -35 °C	24 -48 hrs
^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50-100	25 -100	>=50 %	colourless	30 -35 °C	24 -48 hrs
Enterococcus faecalis ATCC 29212(00087*)	>=10^3	0	0 %		30 -35 °C	24 -48 hrs
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	>=10^3	0	0 %		30 -35 °C	24 -48 hrs
Candida albicans ATCC 10231 (00054*)	50 -100	25 -100	>=50 %	colourless	30 -35 °C	24 -48 hrs

Saccharomyces cerevisiae ATCC 9763(00058*)	50 -100	0 -10	0 -10 %	cream	30 -35 °C	24 -48 hrs

Key :- (*) Corresponding WDCM numbers

(#) Formerly known as *Enterobacter aerogenes* ^ Formerly known as *Pseudomonas aeruginosa*

Storage and Shelf Life

Store between 10-30°C in tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

Reference

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