Technical Information

Medium 10. Levin Eosin- Methylene Blue Agar

Product Code: DM 1022M

Application:-Levin Eosin Methylene Blue Agar Medium is recommended for the isolation, enumeration and differentiation of members of *Enterobacteriaceae* in accordance with Indian Pharmacopoeia.

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

Principle & Interpretation

Levin formulated Levin Eosin Methylene Blue Agar which was [1, 2] 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 Indian Pharmacopoeia [3, 4, 5, 6].
Eosin-Y and methylene blue make 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. Some gram-positive bacteria such as faecal Streptococci, yeasts grow on this medium and form pinpoint colonies. Essential nutrients and growth factors is provided by pancreatic digest of gelatin. Phosphates act 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. A positive dentification 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.

Methodology

Suspend 37.46 grams of powder media in 1000 ml purified/distilled water. Shake well & heat 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.

Quality Control

Physical 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.
Reaction: Reaction of 3.74% w/v aqueous solution at 25^{0}C pH: 7.1-0.2
pH Range
6.90-7.30
Cultural Response/Characteristics
DM 1022M: Growth Promotion is carried out in accordance with IP. Cultural response was observed after an incubation at 36-38°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation temperature	Incubation period

Escherichia coli *ATCC 8739*	50 -100	25 -100	>=50 %	blue-black colonies with metallic sheen	36 -38 °C	18 -24 hrs
Escherichia coli *NCTC 9002*	50 -100	25 -100	>=50 %	blue-black colonies with metallic sheen	36 -38 °C	18 -24 hrs
Additional Microbiological testing						
Escherichia coli *ATCC 25922*	50 -100	25 -100	>=50 %	blue-black colonies with metallic sheen	36 -38 °C	18 -24 hrs
Enterobacter aerogenes *ATCC 13048*	50 -100	25 -100	>=50 %	pink to red	36 -38 °C	18 -24 hrs
Salmonella Typhimurium *ATCC 14028*	50 -100	25 -100	>=50 %	colourless	36 -38 °C	18 -24 hrs
Pseudomonas aeruginosa *ATCC 9027*	50 -100	25 -100	>=50 %	colourless	36 -38 °C	18 -24 hrs
Enterococcus faecalis *ATCC 29212*	>=10^3	0	0%		36 -38 °C	18 -24 hrs
Staphylococcus aureus *ATCC 6538*	>=10^3	0	0%		36 -38 °C	18 -24 hrs
Candida albicans *ATCC 10231*	50 -100	25 -100	>=50 %	colourless	36 -38 °C	18 -24 hrs
Saccharomyces cerevisiae *ATCC 9763*	50 -100	0 -10	0 -10 %	cream	36 -38 °C	18 -24 hrs

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

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. Indian Pharmacopoeia, 2007, Govt. of India, Ministry of Health and Family Welfare, New Delhi.
7. Weld J. T., 1952, Arch. Dermat. Syph., 66:691.
8. Weld J. T., 1953, Arch. Dermat. Syph., 67(5):433.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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