

TM 2159 – LEVINE EOSIN-METHYLENE BLUE AGAR MEDIUM (as per USP)

INTENDED USE

For the isolation, enumeration and differentiation of members of *Enterobacteriaceae*.

PRODUCT SUMMARY AND EXPLANATION

Levine Eosin Methylene Blue Agar Medium was developed by Levine 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.

Weld 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.

COMPOSITION

Ingredients	Gms / Ltr
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

PRINCIPLE

This medium consists of Eosin-Y and methylene blue which 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.

INSTRUCTION FOR USE

- Dissolve 37.46 grams in 1000 ml purified/distilled water.
 - Heat to boiling to dissolve the medium completely.
 - Sterilize by autoclaving at 15 psi 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 photo-oxidation.

QUALITY CONTROL SPECIFICATIONS



Appearance of Powder : Light pink to purple homogeneous free flowing powder.
Appearance of prepared medium : Reddish purple with greenish cast coloured opalescent gel with finely dispersed precipitate forms in Petri plates.
pH (at 25°C) : 7.1 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar and fungal growth on Sabouraud Dextrose Agar.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony	Incubation Temperature	Incubation Period
<i>Escherichia coli</i>	8739	50-100	Good-luxuriant	≥50%	Blue-black colonies with metallic sheen	30-35°C	24-48 Hours
<i>Escherichia coli</i>	25922	50-100	Good-luxuriant	≥50%	Blue-black colonies with metallic sheen	30-35°C	24-48 Hours
<i>Enterobacter aerogenes</i>	13048	50-100	Good-luxuriant	≥50%	Pink to red	30-35°C	24-48 Hours
<i>Salmonella Typhimurium</i>	14028	50-100	Good-luxuriant	≥50%	Colourless	30-35°C	24-48 Hours
<i>Pseudomonas aeruginosa</i>	9027	50-100	Good-luxuriant	≥50%	Colourless	30-35°C	24-48 Hours
<i>Enterococcus faecalis</i>	29212	≥10 ³	Inhibited	0%	-	30-35°C	24-48 Hours
<i>Staphylococcus aureus</i>	6538	≥10 ³	Inhibited	0%	-	30-35°C	24-48 Hours
<i>Candida albicans</i>	10231	10-100	Good-luxuriant	≥50%	Colourless	30-35°C	24-48 Hours
<i>Saccharomyces cerevisiae</i>	9763	10-100	None-poor	0-10%	Cream	30-35°C	24-48 Hours

PACKAGING:



In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.













Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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.

 GMP Good Manufacturing Practices Certified	 IVD For In Vitro Diagnostic Use	 QTY. Quantity	 LOT/ B. NO. Lot / Batch Number	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 EC REP Authorized Representative <small>MedNet GmbH Barkstrasse 10, 48163 Muenster, Germany</small>	 European Conformity	 QR Code	 Consults Instructions for Use	 Best Before

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
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