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EMB Agar, Levine (Levine Eosin Methylene Blue Agar) (DM094U)

Intended Use

EMB Agar, Levine (Levine Eosin Methylene Blue Agar) (DM094U) is recommended for isolation, enumeration and differentiation of members of *Enterobacteriaceae* in compliance with USP.

Product Summary and Explanation

The use of eosin and methylene blue dyes for the differentiation of enteric microorganisms was primarily described by Holt-Harris and Teague. Further, Levine described a modification of their formulation which he claimed gave better differentiation between what are now referred to as *Escherichia* and *Enterobacter* species.[1,2] This medium can also be used 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] The use of Levine EMB Agar, with added Chlortetracycline hydrochloride, for the rapid identification of *Candida albicans* in clinical specimens was developed by Weld.[7,8]

Principles of the Procedure

EMB Agar, Levine (Levine Eosin Methylene Blue Agar) contains pancreatic digest of gelatine which is a source of nitrogen and is an essential growth factor. Phosphates act as good buffering agent. 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 also play a role in differentiating between lactose fermenters and lactose non-fermenters due to the presence or absence of dye uptake in the bacterial colonies. 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. *E.coli* forms colonies with green metallic sheen, indicating strong lactose fermentation.

Formula / Liter

Ingredients	Gms / Liter
Pancreatic digest of gelatine	10.00
Dibasic potassium phosphate	2.00
Lactose	10.00
Eosin – Y	0.40
Methylene blue	0.065
Agar	15.00
Final pH: 7.1 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Store the medium away from light to avoid photooxidation.

Directions

1. Suspend 37.46 grams of medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. AVOID OVERHEATING.

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5. 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.
6. Mix well before pouring into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Light pink to purple homogeneous free flowing powder
Prepared Medium	Reddish purple with greenish cast coloured opalescent gel with finely dispersed precipitate forms in Petri plates
Reaction of % Solution	Not Applicable
Gel Strength	Firm, comparable with 1.5% agar gel

Expected 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.

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation Temperature	Incubation period
	Test for specified microorganism						
1.	*Escherichia coli ATCC 8739*	50 - 100	25 -100	>=50 %	blue-black colonies with metallic sheen	30 - 35 °C	24 - 48 hrs
	Additional Microbiological testing						
2.	*Escherichia coli NCTC 9002*	50 - 100	25 -100	>=50 %	blue-black colonies with metallic sheen	30 - 35 °C	24 - 48 hrs
3.	*Escherichia coli ATCC 25922*	50 - 100	25 - 100	>=50 %	blue-black colonies with metallic sheen	30 - 35 °C	24 - 48 hrs
4.	*Enterobacter aerogenes ATCC 13048*	50 - 100	25 - 100	>=50 %	pink to red	30 - 35 °C	24 - 48 hrs
5.	*Salmonella Typhimurium ATCC 14028*	50 - 100	25 - 100	>=50 %	colourless	30 - 35 °C	24 - 48 hrs
6.	*Pseudomonas aeruginosa ATCC 9027*	50 - 100	25 - 100	>=50 %	colourless	30 - 35 °C	24 - 48 hrs
7.	*Enterococcus faecalis ATCC 29212*	>=10^3	0	0 %	--	30 - 35 °C	24 - 48 hrs
8.	*Staphylococcus aureus ATCC 6538*	>=10^3	0	0 %	--	30 - 35 °C	24 - 48 hrs
9.	*Candida albicans ATCC 10231*	50 - 100	25 - 100	>=50 %	colourless	30 - 35 °C	24 - 48 hrs
10.	*Saccharomyces cerevisiae ATCC 9763*	50 -100	0 - 10	0 -10 %	cream	30 - 35 °C	24 - 48 hrs

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The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Use standard procedures to obtain isolated colonies from specimens.
2. A non-selective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.
3. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours.
4. If negative after 24 hours, reincubate an additional 24 hours. Refer to appropriate references for standard test procedures.

Results

1. *E.coli* forms colonies with green metallic sheen, indicating strong lactose fermentation, whereas colonies of *Salmonella* and *Shigella*, as lactose non-fermenters, appear colorless, transparent or amber.
2. Colonies of *Enterobacter species* are observed as pink to red.
3. Refer to appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. 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.
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : EMB Agar, Levine (Levine Eosin Methylene Blue Agar)
Product Code : DM094U
Available Pack sizes : 500gm

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.

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Further Information

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