PRODUCT SPECIFICATION SHEET

EMB Agar, Levine / Eosin Methylene Blue Agar, Levine (DM094)

Intended Use

EMB Agar, Levine (DM094) is recommended for the isolation, enumeration and differentiation of members of *Enterobacteriaceae* .

Product Summary and Explanation

Levine EMB Agar 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 [3,4,5]. Weld[6,7] proposed the use of Levine EMB Agar, with added Chlortetracycline hydrochloride, for the rapid identification of *Candida albicans* in clinical specimens. 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. Methylene blue and Eosin-Y inhibit gram-positive bacteria to a limited degree. These dyes serve as differential indicators and help to differentiate between lactose-fermenters and non-fermenters in EMB Agar, Levine.

Principles of the Procedure

Peptic digest of animal tissue provides carbon, nitrogen, and other essential growth nutrients in the medium. Lactose and sucrose are the fermentable carbohydrates which are the sources of energy. Eosin-Y and methylene blue serve as differential indicators in response to the fermentation of lactose by microorganisms. Phosphate buffers the medium. The ratio of eosin-Methylene blue is adjusted to approximately 6:1. These indicators also serve to inhibit the growth of most Gram positive organisms to a limited degree. Coliforms produce blue-black colonies due to the taking up of an eosin-methylene blue dye complex by the bacterial cells when the pH drops. *Salmonella* and *Shigella* colonies are colorless or have a transparent amber color. *Escherichia coli* colonies may show a characteristic green metallic sheen due to the rapid fermentation of lactose. Some gram-positive bacteria, such as fecal streptococci, staphylococci and yeasts, will grow on this medium and usually form pinpoint colonies. A number of non-pathogenic, lactose-nonfermenting gram-negative bacteria will grow on this medium and must be distinguished from the pathogenic bacterial strains by additional biochemical tests.

Formula / Liter

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.00
Lactose	10.00
Dipotassium Phosphate	2.00
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	

PRODUCT SPECIFICATION SHEET

Precautions

1. For Laboratory Use only.
2. IRRITANT, mainly irritating to eyes, respiratory system, and skin. Handle in accordance with good laboratory hygiene and safety practice. Wash hands before breaks and at the end of workday. To protect, use safety glasses and gloves during handling.
3. Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Avoid breathing dust.
4. Do not let product enter drains.
5. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 37.46 g of the medium in one liter of purified water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. AVOID OVERHEATING.
4. Cool to 45-50°C and shake the medium in order to oxidize the methylene blue (i.e. to restore its blue colour) and to suspend the flocculent precipitate which is an essential part of the medium.
5. If EMB Agar is inoculated on the same day, it may be used without autoclave sterilization.
6. Test samples of the finished product for performance using stable, typical control cultures.
7. Store the medium away from light to avoid photo-oxidation

Quality Control Specifications

Dehydrated Appearance	Light pink to purple, homogeneous, free flowing powder
Prepared Medium	Reddish purple coloured, opalescent gel with greenish cast and finely dispersed precipitate forms in Petri plates
Reaction of 3.75% Solution	pH 7.1 ± 0.2 at 25°C
Gel Strength	Firm, compared to 1.5% agar gel

Expected Cultural Response: Cultural response on EMB Agar, Levine observed after incubation at 35 ± 2°C for 24-48 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery %	Colour of colony
1.	*Enterobacter aerogenes ATCC 13048*	50-100	good	40-50%	Pink-red without sheen
2.	*Escherichia coli ATCC 25922*	50-100	luxuriant	>=50%	blue-black with metallic sheen
3.	*Escherichia coli NCTC 9002*	50-100	luxuriant	>=50%	blue-black with green metallic sheen
4.	*Escherichia coli ATCC 8739*	50-100	luxuriant	>=50%	blue-black with green metallic sheen
5.	*Candida albicans ATCC 10231*	50-100	luxuriant (incubated in 10% CO_2)	>=50%	colourless
6.	*Enterococcus faecalis ATCC 29212*	50-100	non-poor	<=10%	colourless
7.	*Pseudomonas aeruginosa ATCC 27853*	50-100	luxuriant	>=50%	colourless

PRODUCT SPECIFICATION SHEET

8.	*Salmonella Typhimurium ATCC 14028*	50-100	luxuriant	>=50%	colourless
9.	*Saccharomyces cerevisiae ATCC 9763*	50-100	non-poor	<=10%	cream
10.	*Staphylococcus aureus ATCC 25923*	50-100	non-poor	<=10%	colourless
11.	*Staphylococcus aureus ATCC 6538*	50-100	non-poor	<=10%	colourless
12.	*Pseudomonas aeruginosa ATCC 9027*	50-100	Luxuriant	<=10%	Colourless

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

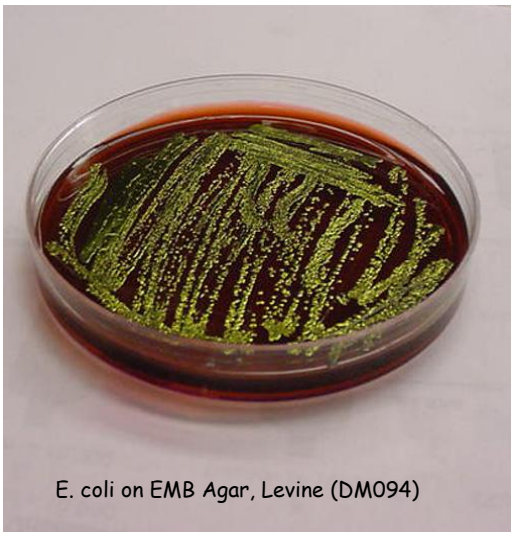
Test Procedure

1. Observe aseptic techniques.
2. Use standard procedures to obtain isolated colonies from specimens.
3. A nonselective 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.
4. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.
5. Confirmatory tests should be further carried out for identification of isolated colonies.

Results

After 18 – 24 hours of incubation at 35 ± 2°C, Typical colonial morphology on EMB Agar is as follows:

Escherichia coli..... Large, blue-black, green metallic sheen
Enterobacter/Klebsiella..... Large, mucoid, blue-black
Proteus..... Large, colorless
Salmonella..... Large, colorless to amber
Shigella..... Large, colorless to amber
Pseudomonas..... Irregular, colorless
Gram-positive bacteria..... No growth to slight growth



E. coli on EMB Agar, Levine (DM094)

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. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Biochemical and serological tests are performed for complete identification.

PRODUCT SPECIFICATION SHEET

Packaging
Product Name : EMB Agar, Levine
Product Code : DM094
Available Pack sizes : 100gm / 500gm

References

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5. Downes F. P and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public
2. Health Association, Washington, D.C.
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7. Weld J. T., 1953, Arch. Dermat. Syph., 67(5):433.
8. Howard B. J., 1994, Clinical and Pathogenic Microbiology, 2nd Ed., Mosby Year Book, Inc

Further Information

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