Technical Information

EMB Agar, Levine

Product Code: DM 1022

Application: - EMB Agar (Levine) is recommended for the isolation, enumeration and differentiation of members of *Enterobacteriaceae*

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Dipotassium phosphate	2.000
Lactose	10.000
Eosin - Y	0.400
Methylene blue	0.065
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Levine EMB Agar was formulated by Levine [1, 2] 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 family *Enterobacter iaceal* by American Public Health Association [3-5]. Weld [6, 7] proposed the use of Levine EMB Agar, with added Chlortetracycline hydrochloride, for the rapid identification of *Candida albicans* from clinical specimens. A positive identification of *Candida albi cans* 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 of colony on the media is variable.

Eosin Y and methylene blue make the medium slightly selective and inhibit the growth of certain gram-positive bacteria. These dyes serve as differential indicators in response to the fermentation of carbohydrates. This helps to differentiate between lactose-ferment ere and non-fermenters in EMB Agar, Levine. The ratio of eosin-methylene blue is adjusted to approximately 6:1. Lactose-fermenting Coliforms produce purplish black colonies due to uptake of methylene blue-eosin dye complex,

when the pH drops. Non-fermenters probably raise the pH of surrounding medium by oxidative de-amination of protein, which solubilizes the methylene blue-eosin complex resulting in formation of colourless colonies [8]. Some strains of *Salmonella* and *Shigella* species do not grow in the presence of eosin and methylene blue.

Peptic digest of animal tissue serves as source of carbon, nitrogen, and other essential growth nutrients. Lactose serves as the source of energy by being the fermentable carbohydrate. Eosin-Y and methylene blue serve as differential indicators. Phosphate buffers the medium. The test sample can be directly streaked on the medium plates. Inoculated plates should be incubated, protected from light. However standard procedures should be followed to obtain isolated colonies. A non-selective medium should be inoculated in conjunction with EMB Agar. Confirmatory tests should be further carried out for identification of isolated colonies.

Methodology

Suspend 37.46 grams of powder media in 1000 ml 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. 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 coloured, opalescent gel with greenish cast and finely dispersed precipitate forms in Petri plates

Reaction

Reaction of 3.75% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH range 6.90-7.30

Cultural Response/ characteristices

DM 1022: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Candida albi cans ATCC 10231	50-100	Luxuriant (incubated in 10% carbon dioxide)	>=50%	colourless
Enterobacter aerogenes ATCC 13048	50-100	good	40-50%	pink-red
Escherichia coli ATCC 25922	50-100	luxuriant	>=50%	blue-black with green metallic sheen
Enterococcus faecalis ATCC 29212	50-100	none-poor	<=10%	colourless
Pseudomonas aeruginosa ATCC 27853	50-100	luxuriant	>=50%	colourless
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	>=50%	colourless
Saccharomyces cerevisiae ATCC 9763	50-100	none-poor	<=10%	cream
Staphylococcus aureus ATCC 25923	50-100	none-poor	<=10%	colourless
Escherichia coli NCTC 9002	50-100	luxuriant	>=50%	blue-black with green metallic sheen
Escherichia coli ATCC 8739	50-100	luxuriant	<=10%	blue-black with green metallic sheen
Staphylococcus aureus ATCC 6538	50-100	none-poor	>=50%	colourless
Pseudomonas aeruginosa ATCC 9027	50-100	luxuriant	>=50%	colourless

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^{o} 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. Greenberg A. E., Trussell R. R. and Clesceri L. S. (Eds.), 1998, Standard Methods ,, for the Examination of Water and Wastewater, 20th ed., APHA, Washington, D.C.
4. Marshall R. (Ed.), 1992, Standard Methods for the Examination of Dairy,, Products, 16th ed., APHA Inc., New York.
5. Downes F. P and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
6. Weld J. T., 1952, Arch. Dermat. Syph., 66:691.
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

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