PRODUCT DATA SHEET

EMB AGAR, LEVINE (ISO 21 150) TM 371

for isolation, enumeration and differentiation of members of Enterobacteriaceae

Composition

Ingredients	Gms/Ltr.
Peptic digest of animal tissue	10.000
Dipotassium phosphate	2.000
Lactose	10.00
Eosin - Y	0.400
Methylene blue	0.065
Agar	15.000

* Dehydrated powder, store in a dry place, in tightly-sealed containers at 24°C and protect from direct Sunlight.

Instructions for Use

Dissolve 37.46gms in 1000 ml of distilled water. Gently heat to boiling with gentle swirling and dissolve the medium completely. Sterilize by autoclaving at 15 psi (121°C) for 15 minutes. Cool to 45 - 50°C and mix well 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.

Appearance: Reddish purple coloured, opalescent gel
PH (at 25°C): 7.1 ± 0.2

Principle

EMB AGAR, LEVINE is used for isolation, enumeration and differentiation of members of Enterobacteriaceae. EMB agar was developed by Holt-Harris and Teague. This formula contains lactose and sucrose with two indicator dyes, Eosin Y and Methylene Blue. Levine modified the formula by removing sucrose and doubling the concentration of lactose. Levine EMB agar 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. Weld proposed the use of Levine EMB Agar, with added Chlortetracycline hydrochloride, for the rapid identification of *Candida albicans* in clinical specimens.

Eosin Y and methylene blue make the medium slightly selective and inhibit certain gram-positive bacteria. These dyes serve as differential indicators in response to the fermentation of carbohydrates. This helps to differentiate between lactose-fermenters and non-fermenters in EMB Agar, Levine. The ratio of eosin-methylene blue is adjusted to approximately 6:1. Coliform produces purplish black colonies due to uptake of methylene blue-eosin dye complex, when the pH drops. The dye complex is absorbed into the colony.

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. Some strains of Salmonella and Shigella species do not grow in the presence of eosin and methylene blue.

Lactose serves as the source of energy by being the fermentable carbohydrate. Peptic digest of animal tissue serves as source of carbon, nitrogen, and other essential growth nutrients. Eosin-Y and methylene blue serve as differential indicators. Phosphate buffers the medium.

Directly streak the test sample 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.

Interpretation

Cultural characteristics observed after inoculating (10^3 CFU/ml) and incubation at 35±2^{0}C for 18 – 24 hours**.**

Microorganisms	ATCC	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli	25922	10^3	Luxuriant	≥ 50%	Black with green metallic sheen
Salmonella typhimurium	14028	10^3	Luxuriant	≥ 50%	Colourless
Enterobacter aerogenes	13048	10^3	Good	40 – 50%	Pink w/o sheen
Staphylococcus aureus	25923	10^3	None-poor	≤ 10%	Whitish

References

1. Levine M., 1918, J. Infect. Dis., 23:43.
2. Levine M., 1921, Bull. 62, Iowa State College Engr. Exp. Station.
3. Marshall R. (Ed.), 1992, Standard Methods for the Examination of Dairy ,, Products, 16th ed., APHA Inc., New York.
4. 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.
5. Weld J. T., 1952, Arch. Dermat. Syph., 66:691.
6. Weld J. T., 1953, Arch. Dermat. Syph., 67(5):433.
7. Downes F. P and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
8. Howard B. J., 1994, Clinical and Pathogenic Microbiology, 2nd Ed., Mosby Year Book, Inc.