TMP 025 – EMB AGAR, LEVINE PLATE

INTENDED USE

For isolation, enumeration and differentiation of Enterobacteriaceae.

PRODUCT SUMMARY AND EXPLANATION

Levine EMB Agar 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. Weld proposed the use of Levine EMB Agar, with added Chlortetracycline hydrochloride, for the rapid identification of 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.

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. Coliforms produce 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.

COMPOSITION

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

PRINCIPLE

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.

INSTRUCTION FOR USE

Either streak, inoculate or surface spread the test inoculum aseptically on the plate.

QUALITY CONTROL SPECIFICATIONS

Appearance	:	Reddish purple coloured medium with greenish cast.
Quantity of Medium	:	25ml of medium in 90mm plates.

pH (at 25°C) : 7.1± 0.2
Sterility Check : Passes release criteria

INTERPRETATION
Cultural response was observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Color of the colony	Incubation Temperature	Incubation Period
Candida albicans	10231	10-100	Good-Luxuriant (Incubated in 10% carbon dioxide)	>=70%	Colourless	35-37°C	24-48 Hours
Enterobacter aerogenes	13048	50-100	Good	40-50%	Pink red	35-37°C	24-48 Hours
Enterococcus faecalis	29212	>=10^3	Inhibited	0%	-	35-37°C	24-48 Hours
Escherichia coli	25922	50-100	Luxuriant	>=70%	Blue- black with metallic sheen	35-37°C	24-48 Hours
Pseudomonas aeruginosa	27853	50-100	Luxuriant	>=70%	Colourless	35-37°C	24-48 Hours
Saccharomyces cerevisiae	9763	10-100	None-poor	0-10%	Cream	35-37°C	24-48 Hours
Salmonella Serotype Typhimurium	14028	50-100	Luxuriant	>=70%	Colourless	35-37°C	24-48 Hours
Staphylococcus aureus	25923	50-100	None-poor	0-10%	Colourless	35-37°C	24-48 Hours

PACKAGING:
Doubled layered packing containing 5 No. of plates with one silica gel desiccant bag packed inside it.

STORAGE

On receipt, store the plates at 15–30 °C. Avoid freezing and overheating. Do not open until ready to use. Prepared plates stored in their original sleeve wrapping until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.
Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or 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.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.

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

***For Lab Use Only**
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