Dehydrated Culture Media
Bases / Media Supplements

Technical Information

EMB Agar, Levine Plate

Product Code: PM 1022

Application: -Recommended for the isolation, enumeration and differentiation of members of Enterobacteriaceae from clinical and non clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Dipotassium hydrogen 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 developed by Levine (6,7) and is used for the differentiation of Escherichia coli and *Klebsiella 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 (2,8,9). Weld (10,11) 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.

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 lactosefermenters 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 deamination of protein, which solubilizes the methylene blue-eosin complex resulting in formation of colourless colonies.

Peptone serves as source of carbon, nitrogen, long chain amino acids, vitamins 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.

Type of specimen

Clinical samples - urine, faeces, oral and vaginal secretions and nail or skin scraping , Foodstuffs; Water samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,10). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. A non-selective medium should be inoculated in conjunction with EMB Agar.
2. Some strains of Salmonella and Shigella species do not grow in the presence of eosin and methylene blue.
3. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
4. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
5. Confirmatory tests should be further carried out for identification of isolated colonies.
6. It is recommended to store the plates ta 24-30°C to avoid minimum condensation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

Appearance
Sterile EMB Agar, Levine in 90mm disposable plates.
pH
6.90- 7.30
Quantity of medium
25 ml of medium in 90 mm disposable plates.
Colour of medium
Reddish purple coloured medium with greenish cast.
Sterility Test
Passes release criteria
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Oragnism	Growth	Inoculum (CFU)	Recovery	Color of Colony
Candida albicans ATCC 10231 (00054*)	50-100	luxuriant (incubated in 10% CO2)	>=50%	colourless
Escherichia coli ATCC 25922 (00013*)	50-100	Good	40-50%	Pink-red
Escherichia coli ATCC 25922 (00013*)	50-100	Luxuriant	>=50%	Blue-black with metallic sheen
Escherichia coli ATCC 8739 (00012*)	50-100	Luxuriant	>=50%	Blue-black with metallic sheen
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	Luxuriant	>=50%	Colourless
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	Luxuriant	>=50%	Colourless
Saccharomyces cerevisiae ATCC 9763	50-100	None-poor	<=10%	Cream
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	None-poor	<=10%	Colourless
Staphylococcus aureus subsp. *aureus* ATCC 8538 (00032*)	50-100	None-poor	<=10%	Colourless

#Key: Formerly known as Aspergillus niger *Corresponding WDCM numbers

Storage and Shelf Life

- On receipt store between 20-30°C.
- Use before expiry date on the label.
- Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2, 4).

Further Reading

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Howard B. J., 1994, Clinical and Pathogenic Microbiology, 2nd Ed., Mosby Year Book, Inc
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Levine M., 1918, J. Infect. Dis., 23:43.
7. Levine M., 1921, Bull. 62, Iowa State College Engr. Exp. Station.
8. Marshall R. (Ed.), 1992, Standard Methods for the Examination of Dairy , Products, 16th ed., APHA Inc., New York.
9. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
10. Weld J. T., 1952, Arch. Dermat. Syph., 66:691.
11. Weld J. T., 1953, Arch. Dermat. Syph., 67(5):433.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and developmentwork carried at **CDH** is true and accurate
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