EMB HiVeg® Agar

MV317

Intended Use:

Recommended for differential isolation of gram negative enteric bacilli from various samples.

Composition**

Ingredients	g / L
HiVeg® peptone	10.000
Dipotassium hydrogen phosphate	2.000
Lactose	5.000
Saccharose (Sucrose)	5.000
Eosin - Y	0.400
Methylene blue	0.065
Agar	13.500
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.96 grams in 1000 ml purified / distilled water. Mix until suspension is uniform. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. **AVOID OVERHEATING.** 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.(If EMB Agar is inoculated on the same day, it may be used without autoclave sterilization). *Precaution : Store the medium away from light to avoid photooxidation*

Principle And Interpretation

EMB HiVeg® Agar is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks. Eosin Methylene Blue (EMB) Agar was originally devised by Holt-Harris and Teague (1) and further modified by Levine (2). The above medium is a combination of the Levine and Holt-Harris and Teague formulae which contains peptone and phosphate as recommended by Levine and two carbohydrates as suggested by Holt-Harris and Teague. Methylene blue and Eosin-Y inhibit gram-positive bacteria to a limited degree. These dyes serve as differential indicators in response to the fermentation of carbohydrates. The ratio of eosin and methylene blue is adjusted approximately to 6:1. Sucrose is added to the medium as an alternative carbohydrate source for typically lactose-fermenting, gram-negative bacilli, which on occasion do not ferment lactose or do so slowly. The coliforms produce purplish black colonies due to taking up of methylene blue-eosin dye complex, when the pH drops. The dye complex is absorbed into the colony. Nonfermenters probably raise the pH of surrounding medium by oxidative deamination of protein, which solubilizes the methylene blue-eosin complex resulting in colourless colonies (3). Some strains of *Salmonella* and *Shigella* species do not grow in the presence of eosin and methylene blue. Further tests are required to confirm the isolates. HiVeg® peptone serves as source of carbon, nitrogen, and other essential growth nutrients. Lactose and sucrose are the sources of energy by being fermentable carbohydrates. 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 HiVeg® Agar. Confirmatory tests should be further carried out for identification of isolated colonies.

Type of specimen

Food samples, Water samples

Specimen Collection and Handling

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

Warning and Precautions

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Due to nutritional variations, certain strains may show poor growth.

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

Light pink to purple homogeneous free flowing powder

Gelling

Firm, comparable with 1.35% 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 3.6% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	good	40-50%	pink, without sheen
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=50%	purple with black centre and green metallic sheen
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good	40- 50%	pink, mucoid
Proteus mirabilis ATCC 25933	50-100	luxuriant	>=50%	colourless
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	>=50%	colourless
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : (*) Corresponding WDCM numbers. (#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. 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 (6,7).

Reference

1.Holt-Harris and Teague,1916, J. Infect. Dis., 18 : 596.

2. Levine, 1918, J. Infect. Dis., 23:43.
3. Howard B.J., 1994, Clinical and Pathogenic Microbiology, 2nd ed., Mosby Year Book, Inc.
4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed.Washington DC:APHA Press; 2023.
6. Isenberg (Eds.), 1992, Clinical Microbiology Procedures Handbook, Vol . 1, American Society for Microbiology, Washington, D.C.
7. 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.

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