

Entamoeba HiVeg[®] Medium

MV077

Intended Use:

Recommended for cultivation of *Entamoeba histolytica*.

Composition**

| Ingredients | g / L |
|-----------------------------------|---------|
| HiVeg [®] infusion No. 1 | 10.8000 |
| HiVeg [®] peptone No. 3 | 5.500 |
| Sodium alpha-glycerophosphate | 3.000 |
| Sodium chloride | 2.700 |
| Agar | 11.000 |
| Final pH (at 25°C) | 7.0±0.2 |

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.0 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Distribute in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Allow tubes to solidify in a slanted position. Cover about half of the slant with fresh sterile horse serum-saline mixture (1:6) and add a 5 mm loopful of rice powder, which has been sterilized in an oven at 160°C for one hour. **SCORCHING OF THE MEDIUM SHOULD BE PREVENTED.**

Principle And Interpretation

Amoebiasis occurs throughout all areas of the world, the causative agent of which is *Entamoeba histolytica*, the only amoeba pathogenic for humans (1,2,3). In nature, the organism exists in the cyst form. It enters the body through contaminated food or water. The organism passes through the stomach as cyst and the trophozoite amoebas emerge in the intestine. *E. histolytica* destroys the tissue of the large intestine, causing lesions, deep ulcers and diarrhea (1). Entamoeba Medium formulated by Cleveland and Sanders (4) and Cleveland and Collier (5), is used for the cultivation of *E.histolytica*.

Entamoeba HiVeg[®] Medium is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks. This medium is highly specific for *E.histolytica*, which grows luxuriantly on this medium. Other intestinal amoebae do not grow readily on this medium. The technique of overlaying the medium with fresh sterile horse serum-saline mixture, as reported by Cleveland and Collier, was reported to be the best method of isolation of *E.histolytica* (6). HiVeg[®] infusion No. 1 and HiVeg[®] peptone No. 3 from provide amino acids and other nitrogenous substances that support growth of *E.histolytica*. Sodium chloride maintains the osmotic balance of the medium and sodium alpha-glycerophosphate acts as a phosphorous source.

Type of specimen

Clinical samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).

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. Other intestinal Amoebae do not grow readily on this medium.

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to brownish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.1% Agar gel.

Colour and Clarity of prepared medium

Dark amber coloured, slightly opalescent gel forms in tubes as slants

Reaction

Reaction of 3.3% w/v aqueous solutions at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for 68-72 hours.

Organism

Growth

Entamoeba histolytica ATCC 30190 luxuriant

Entamoeba invadens ATCC 30016 luxuriant

Entamoeba moskkovskii ATCC 30042 luxuriant

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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 (7,8).

Reference

1. Alcamo I. E., 2001, Fundamentals of Microbiology, 6th Ed., Jones and Bartlett Publishers.
2. Bruckner D. A., 1992, Amebiosis. Clin. Microbiol., Rev. 5: 356-369.
3. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
4. Cleveland and Sanders, 1930, Arch. Protistenkunde, 70:223.
5. Cleveland and Collier, 1930, Am. J. Hyg., 12:606.
6. Sixth Annual Year Book 1935-36, P. 130, Suppl., Am. J. Pub. Health, 1936, 26, No. 3.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. 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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