



Entamoeba Medium

M077

Entamoeba Medium is used for cultivation of *Entamoeba histolytica* .

Composition**

Ingredients	Gms / Litre
Liver, infusion from	272.000
Proteose peptone	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 grams in 1000 ml 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. 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 (1-3). 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* .

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). Proteose peptone and infusion from liver provide amino acids and other nitrogenous substances that support growth of *E. histolytica* . Sodium chloride maintains the osmotic balance of the medium and sodium a-glycerophosphate acts as a phosphorous source.

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

M077: Cultural characteristics observed after an incubation at 25-30°C for 68-72 hours.

Organism

Growth

Cultural Response

Entamoeba histolytica ATCCluxuriant
30190
Entamoeba invadens ATCC luxuriant
30016
Entamoeba moskkovskii luxuriant
ATCC 30042

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Alcamo I. E., 2001, Fundamentals of Microbiology, 6th Ed., Jones and Bartlett Publishers.
2. 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.
3. Bruckner D. A., 1992, Amebiosis. Clin. Microbiol., Rev. 5: 356-369.
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.

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