Liver-Veal-Agar Base, Modified

M1872

Liver-Veal-Agar, Modified is recommended for isolation of *Clostridium botulinum* in accordance with FDA BAM,1998.

Composition**

Ingredients	Gms / Litre
Liver infusion from	50.000
Veal, infusion from	500.000
Proteose peptone	20.000
Peptone special	1.300
Tryptone	1.300
Dextrose	5.000
Starch soluble	10.000
Sodium chloride	5.000
Casein isoelectric	2.000
Sodium nitrate	2.000
Gelatin	20.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 97 grams in 1000 ml warm distilled water. Heat to boiling to dissolve the medium completely, and sterilize at15 lbs (121ºC) for 15min .Cool to 45-50 ºC. Aseptically add 80 ml Egg yolk emulsion,50% (FD045F) . Mix well and pour into sterile Petri dishes.

Principle And Interpretation

Anaerobic bacteria live in an oxygen-free environment. Some of the anaerobic bacteria die in presence of oxygen while others fail to grow and multiply (1). Liver Veal Agar Base, Modified (M1872) is a modification of the medium formulated by Spray, 1936 (2). It is recommended by the FDA Bacteriological Analytical Manual (BAM) (3) for the growth of anaerobic organisms especially *Clostridium botulinum* . This may also be used in supplementation with 50% egg yolk (FD045F) (3,4). *Clostridium botulinum* is an anaerobic, rod-shaped spore forming bacterium that produces a protein with characteristic neurotoxicity. Under certain conditions, these organisms may grow in foods producing highly dangerous botulinum toxin(s). Botulinum toxin has been classified into botulinum A, botulinum B upto G. Among this all except F and G are known to cause animal botulism. Different strains are classified through antigenic characterization using appropriate antitoxins. They are also differentiated into general groups on the basis of cultural, biochemical, and physiological characteristics.

Both the infusions, peptones, casein enzymic hydrolysate and gelatin serve as sources of carbon, nitrogen, amino acids and various vitamins. Dextrose serves as the energy source. Starch enhances growth of anaerobic bacteria. Sodium chloride maintains the osmotic equilibrium of the medium. Agar acts as the solidifying agent.

According to the FDA BAM protocol, suspected samples after preliminary examination is proceeded for enrichment of the organism. 1-2 g or 1-2 ml of samples after removing the dissolved oxygen content are inoculated into Cooked meat medium (M149) and Tryptone Peptone Glucose Yeast Extract Broth Base w/o Trypsin (M969) and incubated at 35°C and 25°C respectively. Upon 5 days of incubation, growth is checked by turbidity, gas production, and digestion of meat particles and different staining procedures of the growth. For isolation of pure cultures, alcohol pretreated samples are inoculated into Liver-Veal-Agar Base, Modified (M1872) and/ or Anaerobic Egg Agar Base (M902F). The luster zones of different types vary often including variation in yellow precipitates. Serological, biochemical and invivo assays are performed to confirm the serotypes.

Quality Control

Appearance
Light yellow to brownish yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Amber coloured clear to slightly opalescent gel forms in Petri plates, may have slight precipitate.

Reaction
Reaction of 9.7% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours with addition of Egg yolk emulsion(under the atmospheric requirement of organism).

Cultural Response

Organism	Growth
Cultural Response	
Clostridium botulinum ATCC 25763	luxuriant
Clostridium tetani ATCC 10709	luxuriant
Neisseria meningitidis ATCC 13090	luxuriant
Streptococcus pneumoniae ATCC 6303	luxuriant

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 6 ed.: Jones and Bartlett Publishers.
2.Spray, R. S. 1936. J. Bacteriol, 32(135).
3.FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, Md. : AOAC International.
4.APHA. 2001. Compendium of Methods for the Microbiological Examination of Foods.F. P Downes and Ito K Ed. Washington, D.C.

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