Anaerobic Egg Agar Base

M902F

Anaerobic Egg Agar Base supplemented with egg yolk emulsion is recommended for detection of *Clostridium botulinum* in foods in accordance with FDA BAM, 1998.

Composition**

Ingredients	Gms / Litre
Yeast Extract	5.000
Tryptone	5.000
Proteose peptone	20.000
Sodium chloride	5.000
Agar	20.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 55 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 80 ml sterile Egg Yolk Emulsion (FD045F). Mix thoroughly before pouring into sterile Petri plates.

Principle And Interpretation

Clostridium species ranked behind *Salmonella* and *Staphylococcus aureus* , has been the third most common etiological agent of food-borne diseases (1). *Clostridium botulinum* is an anaerobic, rod-shaped spore forming bacterium that produces a botulinum toxin with characteristic neurotoxicity (2). *Clostridium* food poisoning results from consumption of contaminated food. Anaerobic Egg Agar Base supplemented with egg yolk emulsion is recommended for detection of *Clostridium botulinum* in foods and also to detect the lipase activity of *Yersinia* sp. in accordance with FDA BAM, 1998 (3). The major virulence factor of *C. botulinum* is botulinum toxin, which is secreted upon invasion of the host gut, which contributes to food poisoning, gastrointestinal illnesses and even death (2).

Casein enzymic hydrolysate and proteose peptone supply amino acids and other complex nitrogenous nutrients. Yeast extract provides essential B-complex vitamins. Egg yolk emulsion (FD045F) is added to the medium by which the lipase and lecithinase activity can be observed. Agar acts as the solidification agent. Sodium chloride maintains the osmotic equilibrium of the cells. *Yersinia* sp. degrades lecithin of egg yolk, forming an insoluble opaque precipitate (4). Lipase breaks down free fats present in the egg yolk causing iridescent sheen to form on the colony surface. Proteolysis is indicated by clear zones in the medium surrounding the growth (5).

Representative samples of food (1-2 g solid or 1-2ml liquid) are enriched in Cooked Meat Media (M149F) up to 5days at 35°C under anaerobic conditions. Vegetative cells are destroyed either by alcohol treatment (3) or by heat treatment wherein an aliquot of 1-2ml is heated at 80°C for 10-15min. These treated cultures are streaked to either Liver-Veal-Agar Base, Modified (M1872) or Anaerobic Egg Agar Base (M902F). Typical *C.botulinum* colonies may be raised or has smooth or rough surfaces. They show spreading and have an irregular edge. On egg yolk medium, *C.botulinum* colonies usually exhibit surface iridescence when examined by oblique light. This luster zone often referred to as a pearl layer, usually extends beyond and follows the irregular contour of the colony. Colonies of *C. botulinum* types C, D, and E are ordinarily surrounded by a wide zone (2-4 mm) of yellow precipitate. Colonies of types A and B generally show a smaller zone of precipitation. Further biochemical tests need to be performed to differentiate it from other species of *Clostridium* .

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium

Basal medium -Light yellow coloured, clear to very slightly opalescent gel. After addition of Egg Yolk Emulsion -Light yellow coloured, opaque gel forms in Petri plates

Reaction

Reaction of 5.5% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

M902F: Cultural characteristics observed with addded Egg Yolk Emulsion, 50% (FD045F) when incubated anaerobically at 35-37ºC for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Lecithinase	Lipase
Cultural Response					
Clostridium perfringens ATCC 12924	50-100	good-luxuriant	>=50%	positive reaction, opaque zone around the colony	negative reaction
Clostridium sporogenes ATCC 11437	50-100	good-luxuriant	>=50%	negative reaction	positive reaction, irridescent sheen on the colony

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 label.

Reference

1.Centre for Disease Control, 1982, CDC Surveillance Summaries, 35:7SS-16SS, 1986.
2.Czeczulin, J. R., Hanna, P. C. and Mcclane, B. 1993. Infect. Immun., 61: 3429-3439.
3.FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
4.Finegold. and Baron. 1986. Bailey and Scott’s Diagnostic Microbiology. 7 ed. St. Louis: The C.V. Mosby Company.
5.Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A. and Yolken, R. H. 2003. Manual of Clinical Microbiology. 8 ed. Washington, D.C: ASM.

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