Anaerobic Egg Agar Base

M902

Anaerobic Egg Agar Base supplemented with egg yolk emulsion is recommended for detection of *Clostridium perfringens* in foods.

Composition**

Ingredients	Gms / Litre
Proteose peptone	20.000
Casein enzymic hydrolysate	5.000
Yeast extract	5.000
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 (FD045). Mix thoroughly before pouring into sterile Petri plates.

Principle And Interpretation

Clostridium perfringens , ranked behind *Salmonella* species and *Staphylococcus* aureus, has been the third most common etiological agent of food-borne disease (1). *Clostridium* species are spore forming, gram-positive rods occurring naturally in soil (2). C. perfringens food poisoning results from eating contaminated food. The major virulence factor of *C. perfringens* is the CPE enterotoxin, which is secreted upon invasion of the host gut, and contributes to food poisoning and other gastrointestinal illnesses (2). C. perfringens cells may lose viability if the suspected food samples are refrigerated, thereby making it difficult to incriminate the organisms in food poisoning outbreaks (3). Anaerobic Egg Agar is one of the media recommended by APHA (4) for detecting *C. perfringens* in foods.

Casein enzymic hydrolysate and proteose peptone supply amino acids and other complex nitrogenous nutrients. Yeast extract provides essential B-complex vitamins. Egg yolk emulsion is added to the medium by which the lipase and lecithinase activity can be observed. Lecithinase of *C. perfringens* degrades lecithin of egg yolk, forming an insoluble opaque precipitate (5). Lipase breaks down free fats present in the egg yolk causing irridescent sheen to form on the colony surface. For the lipase reaction, plates may be kept upto a week for incubation (5). Proteolysis is indicated by clear zones in the medium surrounding the growth (6).

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

M902: Cultural characteristics observed with addded Egg Yolk Emulsion (FD045) when incubated anaerobically,at 35-37ºC for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Lecithinase	Lipase
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

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4. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
5. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Company, St. Louis.
6. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

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