Egg Yolk Agar Base, Modified

M1043

Egg Yolk Agar Base, Modified is recommended for identification of anaerobic bacteria by means of their egg yolk reaction.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	15.000
Papaic digest of soyabean meal	5.000
Yeast extract	5.000
Sodium chloride	5.000
L-Cystine	0.400
Hemin	0.005
Vitamin K1	0.010
Agar	20.000
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50.41 grams in 900 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 50-55°C and aseptically add 100 ml Egg Yolk Emulsion (FD045) (or add 10 ml of sterile egg yolk emulsion (FD045) per 90 ml of medium). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Clostridium perfringens food poisoning is one of the most common types of human food borne illness (1). The foods usually involved are cooked meat or poultry products containing large numbers of viable cells. A heat- labile enterotoxin produced only by sporulating cells (2) induces the major symptoms of diarrhea in perfringens poisoning.

Egg Yolk Agar Base, Modified is based on McClung and Toabe Agar Base (3) for isolation and detection of *C. perfringens* . In Egg Yolk Agar Base, Modified, CDC Anaerobe Agar is used as a base to prepare the medium. CDC Anaerobe Agar is a non-selective, highly enriched medium for the cultivation of obligate anaerobes, developed by Center for Disease Control (CDC) (4). The medium is made suitable for detection of lipase and lecithinase activity by the addition of egg yolk emulsion (5-7).

Casein enzymic hydrolysate and papaic digest of soyabean meal provide the essential nutrients along with carbonaceous and nitrogenous substances. Yeast extract supplies B-complex nutrients. Ssdium chloride maintains the osmotic equilibrium. L-cystine is an amino acid which also acts as a reducing agent. Vitamin K1 and hemin help to enhance the growth of anaerobic organisms. Organisms producing lecithinase break down lecithin present in the egg yolk emulsion producing an insoluble opaque precipitate around the colonies. Lipase-producing organisms break down free fatty acids (in the egg yolk emulsion) forming an iridescent sheen on the surface of the colonies. Lipase activity may be delayed, therefore plates should not be discarded as negative before incubation for a week. Proteolytic activity is seen as clear zones around the colonies (6). The media should be directly inoculated with the test specimen. Prior to inoculation, the media plates should be reduced by placing in an anaerobic jar for 18-24 hours.

An enrichment broth should be simultaneously inoculated with the test sample to detect small number of anaerobic organisms. Standard procedures for the isolation of organism should be referred. Incubation should be carried out for 18-48 hours and continued for 7 days.

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 : Medium amber coloured clear to slightly opalescent gel After addition of egg yolk emulsion (FD045)- Yellow coloured opaque gel forms in Petri plates.

Reaction
Reaction of 5.04% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH
7.30-7.70

Cultural Response
M1043: Cultural characteristics observed with added Egg yolk emulsion (FD045),after an incubation at 35-37°C for 48-72 hours when incubated anaerobically. (*Plates should be incubated up to 7 days before regarding them as negative)

Organism	Inoculum (CFU)	Growth	Recovery	Lecithinase	Lipase activity*	Proteolytic activity
Clostridium perfringens ATCC 12924	50-100	good-luxuriant	>=50%	positive,opaque zone around the colony	negative,no irridescent sheen on the colony surface and medium	negative,no clear zone surrounding colonies
Fusobacterium necrophorum ATCC 25286	50-100	good-luxuriant	>=50%	negative reaction	positive, irridescent sheen on the colony surface and medium	negative,no clear zone surrounding colonies
Clostridium sporogenes ATCC 11437	50-100	good-luxuriant	>=50%	negative reaction	positive, irridescent sheen on the colony surface and medium	positive ,clear zone surrounding colonies

Storage and Shelf Life
Store below 30°C in tightly closed container and use the prepared medium as fresh as possible. Use before expiry date on the label.

Reference
1. Labbe R., 1989, Clostridium perfringens, In Foodborne Bacterial Pathogens Ed., Doyle M. P., P.191, Marcel Dekker, New York , N.Y.,
2. Duncan C. L., 1973, A. J. Bacteriol., 113:932
3. McClung and Toabe, 1947, J. Bacteriol., 53:139
4. Dowell, Lombard, Thompson and Armfield, 1977, Media for Isolation, Characterization and Identification of Obligately Anaerobic Bacteria, CDC Laboratory Manual, Center for Disease Control, Atlanta, Ga.
5. Dowell and Hawkins, 1987, Laboratory Methods in Anaerobic Bacteriology, CDC Laboratory Manual, HHS Publication No. (CDC) 87-8272, Centers for Disease Control, Atlanta, Ga.
6. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
7. Baron E. J., Peterson and Finegold S. M., Bailey & Scotts Diagnostic Microbiology, 9th Ed., 1994, Mosby-Year Book, Inc., St. Louis Mosby Co., St. Louis.

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