Technical Information

Egg Yolk Agar Base

Product Code: DM 1808

Application: - Egg Yolk Agar Base is used for isolation and identification of Clostridia and other anaerobic microorganisms.

Composition**

Ingredients	Gms / Litre
Proteose peptone	40.000
Disodium phosphate	5.000
Monopotassium phosphate	1.000
Sodium chloride	2.000
Magnesium sulphate	0.100
Glucose	2.000
Hemin	0.005
Agar	25.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Clostridium perfringens food poisoning is the Commonest types of human food borne illness [1]. The foods usually responsible 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 after consuming the food.

Egg Yolk Agar Base has slight modification [3] of McClung Toabe Agar Base [4] used for isolation and detection of Clostridium perfringens. Egg Yolk Agar Base differs from the original formula by the inclusion of hemin.

Proteose peptone provides the essential nutrients along with carbonaceous and nitrogenous substances. Phosphates buffer the medium whereas sodium chloride maintains the osmotic equilibrium. Magnesium sulphate serves as a source of divalent cations along with sulphates. Glucose serves as a source of energy. Hemin helps 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 [5]. The media should be directly inoculated with the test specimen. Prior to inoculation, 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.

Methodology

Suspend 75.10 grams of powder media in 900 ml distilled water. Shake well & heat to dissolve the medium completely. Dispense in 90 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and add 10 ml of sterile egg yolk emulsion (MS2045) per 90 ml of medium. Mix well and pour into sterile Petri plates.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.5% Agar gel.

Colour and Clarity of prepared medium

Basal medium: Medium amber coloured, clear to slightly opalescent gel After addition of egg yolk emulsion (MS2045): Yellow coloured opaque gel forms in Petri plates

Reaction

Reaction of 7.5% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH range 7.40-7.80

Cultural Response/ characteristices

DM 1808: Cultural characteristics observed with added Egg yolk emulsion (MS2045), 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*	Protcolytic activity
Bacteroides fragilis ATCC 25285	50-100	good-luxuriant	>=50%	negative reaction	negative reaction,no irridescent sheen on the colony surface and medium	negative,no clear zone surrounding colonies
Clostridium botulinum ATCC 25763	50-100	good-luxuriant	>=50%	negative reaction	negative reaction,no irridescent sheen on the colony surface and medium	positive, clear zone surrounding colonies
Clostridium butyricum ATCC 13732	50-100	good-luxuriant	>=50%	negative reaction	negative reaction,no irridescent sheen on the colony surface and medium	positive clear zone surrounding colonies
Clostridium perfringens ATCC 12924	50-100	good-luxuriant	>=50%	positive,opaque zone around the colony	negative reaction,no 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

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8^{o} in sealable plastic bags for 2-5 days.

Further Reading

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. Atlas R. M., 2004, Handbook of Microbiological Media, 3rd Ed., CRC Press.
4. McClung and Toabe, 1947, J. Bacteriol., 53:139
5. 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.

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