Clostridium difficile Mannitol Taurocholate Broth Base

M1976

It is used for cultivation of *Clostridium difficile* from certain clinical specimens.

Composition**

Ingredients	Gms / Litre
Proteose peptone	40.000
Disodium hydrogen phosphate	5.000
Potassium dihydrogen phosphate	1.000
Sodium chloride	2.000
Magnesium sulfate	0.100
Mannitol	6.000
Neutral red	0.030
Sodium taurocholate	1.000
Cysteine	0.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 55.63 grams in 1000 ml distilled water. Heat if necessary to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 1 vial of Clostridium difficile Selective Supplement (FD320). Mix well and dispense into sterile tubes.

Principle And Interpretation

Clostridium difficile Mannitol Taurocholate Broth Base is used for the primary isolation of *C. difficile* from faecal specimens(1). The spectrum of disease caused by *Clostridium difficile* (a pathogenic *Clostridium* affecting the bowel) ranges from pseudomembranous colitis (PMC) through antibiotic associated colitis (AAC). It also includes chronic inflammatory bowel diseases, post-operative diarrhoea and non-antibiotic associated diarrhoea (2). Smith and King (3) first reported the presence of *C. difficile* in human infections.

The medium composition is designed so as to obtain luxuriant growth of *C. difficile* . The selective agents D-cycloserine and cefoxitin used in the medium inhibit the growth of majority of *Enterobacteriaceae* and also Enterococcus faecalis , gram-negative anaerobic bacilli and *Clostridium* species other than *C. difficile* , which may be found abundantly in faecal samples. Proteose peptone provides essential growth factors and trace nutrients. Mannitol is the fermentable carbohydrate, fermentation of which leads to acid production, detected by neutral red indicator. Taurocholate and lysozyme are added as spore germination stimulators. Inorganic salts supply the necessary growth requirements. Sodium chloride maintains the osmotic equilibrium.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Red coloured clear solution in tubes.

Reaction
Reaction of 5.56% w/v aqueous solution at 25°C. pH : 7.3±0.2

Cultural Response
Cultural characteristics observed under anaerobic condition with added Clostridium difficile Selective Supplement(FD320) after an incubation at 35-37°C for 48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Acid
Cultural Response			
Clostridium difficile ATCC 11204	50-100	luxuriant	positive reaction,yellow colour
Clostridium sporogenes ATCC 11437	>=10^3	inhibited	negative reaction, no colour change
Clostridium perfringens ATCC 12924	>=10^3	inhibited	negative reaction, no colour change
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	negative reaction, no colour change
Bacteroides fragilis ATCC 25285	>=10^3	inhibited	negative reaction, no colour change
Streptococcus faecalis ATCC 29212	>=10^3	inhibited	negative reaction, no colour change
Proteus mirabilis ATCC 25933	>=10^3	inhibited	negative reaction, no colour change

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.Holdeman,L.V.,F.P.Cato and W.E.C.Moore.1977.Anaeobe Laboratory Manual. Virginia Polytechnic Institute and State University. Blacksburg, VA24061.
2. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 14th Ed., Churchill Livingstone.
3. Smith L. D. S. and King E. O., 1962, J. Bacteriol., 84:65.

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