

Technical Data

Clostridium difficile Mannitol Taurocholate Broth base

M1976

Intended Use

Recommended used for cultivation of Clostridium difficile from certain clinical specimens.

Composition**		
Ingredients	g / L	
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	
L-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 purified / 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 LCC 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.

Type of specimen

Clinical sample - faeces

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Some strains of C.difficile may show poor growth due to nutritional variations.

2. Complete identification to be done using biochemical characteristics and serological tests on pure culture.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry periodwhen stored at recommended temperature.

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 LCC Selective Supplement (FD320) after an incubation at 35-37°C for 48 hours.

Organism	Inoculum (CFU)	Growth	Acid
<i>Clostridium difficile</i> ATCC 9689	50-100	luxuriant	positive reaction, yellow colour
Clostridium difficile ATCC 700057	50-100	luxuriant	positive reaction, yellow colour
Clostridium difficile ATCC BAA 1870	50-100	luxuriant	positive reaction, yellow colour
Clostridium sporogenes ATCC 11437	>=10 ⁴	inhibited	negative reaction, no colour change
Clostridium perfringens ATCC 12924	>=10 ⁴	inhibited	negative reaction, no colour change
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10 ⁴	inhibited	negative reaction, no colour change
<i>Bacteroides fragilis</i> ATCC 25285	>=10 ⁴	inhibited	negative reaction, no colour change
Enterococcus faecalis ATCC 29212 (00087*)	>=10 ⁴	inhibited	negative reaction, no colour change
Proteus mirabilis ATCC 25933	>=10 ⁴	inhibited	negative reaction, no colour change

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

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.

4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

5.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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