Clostridium Difficile Agar Base

M836

Clostridium Difficile Agar Base with supplement is used for cultivation of *Clostridium difficile* from food and certain pathological specimens.

Composition**

Ingredients	Gms / Litre
Proteose peptone	40.000
Disodium phosphate	5.000
Monopotassium phosphate	1.000
Magnesium sulphate	0.100
Sodium chloride	2.000
Fructose	6.000
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 34.55 grams in 500 ml distilled water. Heat gently to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C. Aseptically add rehydrated contents of 1 vial of Clostridium Difficile Supplement (FD010) together with 7% (v/v) defibrinated Horse blood or Sheep blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

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 (1). Smith and King (2) first reported the presence of *C. difficile* in human infections. George et al (3) recommended the use of a fructose-containing medium with egg yolk for the isolation of *C. difficile* from faecal specimens. The medium was made inhibitory to the accompanying flora by the addition of the selective agents namely, D-cycloserine and cefoxitin.

This medium does not contain neutral red indicator, as in the original formulation, as it is recommended for use with sheep or horse blood (3). Clostridium Difficile Agar Base is used for the primary isolation of *C. difficile* from faecal specimens.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* , Staphylococci, gram-negative anaerobic bacilli and *Clostridium* species other than *C. difficile* , which may be found abundantly in faecal samples. Addition of 7% v/v horse blood to the base increases the recovery of *C. difficile* and also increases its colony size.

Spread a part of the faecal sample on the medium to obtain isolated colonies. Incubate the plates anaerobically at 37°C for 18 - 48 hours. *C. difficile* forms grayish white, irregular, raised and opaque colonies, 4-6 mm in diameter, after 48 hours. Typical gram stain morphology of *C. difficile* may not be seen in colonies taken from this medium due to the presence of antibiotics. Subculture on Blood Agar (M073) to obtain characteristic morphology. *C. difficile* colonies will not exhibit the typical fluorescence and colour of colony on this medium whereas other Clostridia can give fluorescence. Therefore, for complete identification and confirmation, other tests like gram staining, morphology, biochemicals, specific cytotoxin and clinical observation should be carried out.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Basal medium: Light amber coloured clear to slightly opalescent gel. After addition 7% v/v defibrinated horse blood: Cherry red coloured, opaque gel forms in Petri plates.

Reaction
Reaction of 6.91% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics observed under anaerobic condition with added Clostridium Difficile Supplement(FD010) and 7% v/v defibrinated horse blood, after an incubation at 35-37°C for 48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cultural Response				
Clostridium difficile ATCC 11204	50-100	good-luxuriant	>=50%	greyish-white
Shigella flexneri ATCC 12022	>=10^3	inhibited	0%	
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	

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. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 14th Ed., Churchill Livingstone.
2. Smith L. D. S. and King E. O., 1962, J. Bacteriol., 84:65.
3. George W. L., Sutter V. L., Citron D., and Finegold S. M., 1979, J.Clin. Microbiol., 9:214

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