Clostridium Difficile Agar Base

M836

Intended Use:

Recommended for selective isolation of *Clostridium difficile* from food and certain pathological specimens.

Composition**

Ingredients	g / L
Proteose peptone	40.000
Disodium hydrogen phosphate	5.000
Potassium dihydrogen 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 purified/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 45-50°C. Aseptically add rehydrated contents of one vial of CC Selective Supplement I (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.

Type of specimen

Clinical samples - Stool sample; Food samples.

Specimen collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. 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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical and serological tests must be carried out for further identification.

Performance and Evaluation

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

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 CC Selective Supplement I (FD010) and 7% v/v defibrinated horse blood, after an incubation at 35-37°C for 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Clostridium difficile ATCC 11204	50-100	good-luxuriant	>=50%	greyish-white
Shigella flexneri ATCC 12022 (00126*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Staphylococcus aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	

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.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
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.
6.Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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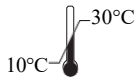
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

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