

# Clostridium Difficile Selective Supplement

## PRODUCT INFORMATION

C041-1g - D-Cycloserine, Crystal, 1g

C041-5g - D-Cycloserine, Crystal, 5g

C091-1g - Cefoxitin, Powder, 1g

C054-250mg - Cefoxitin Sodium, Powder, 250mg

C054-1g - Cefoxitin Sodium, Powder, 1g

C054-5g - Cefoxitin Sodium, Powder, 5g

## DESCRIPTION

*Clostridium difficile* Agar Base with *Clostridium difficile* Selective Supplement is a medium for the isolation of *Clostridium difficile*.

## BACKGROUND

Cycloserine is an antibiotic effective against *Mycobacterium tuberculosis*.

Cefoxitin is a cephamycin antibiotic developed by Merck & Co., Inc., often grouped with the second-generation cephalosporins.

### Mechanism of action

Cycloserine's terminal two amino acid residues of the murein precursor lipid II consist of D-alanine, which is produced by the enzyme alanine racemase; the two residues are joined by D-alanine ligase. Both enzymes are competitively inhibited by cycloserine

## APPLICATION IN CLOSTRIDIUM DIFFICILE AGAR BASE

*Clostridium difficile* was first isolated in 1935 by Hall and O'Toole who proposed the name 'difficile' because it was very difficult to isolate. In 1940 Snyder isolated *Clostridium difficile* from infants aged 10 weeks to 1 year. No further isolations were reported until 1960, when the organism was cultured by McBee from the intestinal contents of a seal, and in 1962 Smith and King reported its presence in human infections.

Toxicogenic isolates of *Clostridium difficile* have been demonstrated to be a major cause of antibiotic-

associated ileo-caecitis in laboratory animals and pseudomembranous colitis in man. Keighley found *Clostridium difficile* was associated with colitis and diarrhoea without pseudomembranous changes after antibiotic therapy following gastrointestinal operations.

Hafiz and Oakley devised a medium for the selective isolation of *Clostridium difficile* based on the observation that the organism has a high tolerance to cresol, which it produces during its growth, and used reinforced clostridial medium plus 0.2% phenol or p-cresol.

George et al in a study of selective media for the routine isolation of *Clostridium difficile* from faecal specimens found this medium was inhibitory compared with growth on blood agar. They recommended the use of a fructose containing nutrient medium plus egg yolk, with D-cycloserine and cefoxitin as selective agents for the isolation of *Clostridium difficile*.

The combination of *Clostridium difficile* Agar Base plus the Culture Media Supplement is based on the formulation proposed by George et al.

The selective agents D-cycloserine (500µg/ml) and cefoxitin (16µg/ml) inhibit growth of the majority of Enterobacteriaceae, as well as *Streptococcus faecalis*, *staphylococci*, Gram-negative non-sporing anaerobic bacilli and *Clostridia* species. (except *Clostridium difficile*) which may be found in large numbers in faecal samples.

Levett, noting reports that some strains of *Clostridium difficile* had low minimum inhibitory concentrations to both cycloserine and cefoxitin, reduced the antibiotic concentrations to 125 µg per ml cycloserine and 4 µg per ml cefoxitin and combined this with alcohol shock to compensate for the reduction in selectivity. *Clostridium difficile* was isolated from all of the 33 faecal specimens plated on to CCFA Medium containing cycloserine and cefoxitin at 250 µg per ml and 8 µg per ml respectively, but from only 25/33 specimens plated on to medium containing 500 µg per ml cycloserine and 16 µg per ml cefoxitin. The specimen should be treated with alcohol before inoculation.

It can be expected that medium containing the lower concentration of antibiotics will yield a greater growth of contaminating organisms if antibiotics are used alone, but Levett reported that there was no difference in the growth of contaminating organisms on plates

containing either concentration of antibiotics following alcohol shock treatment of the specimen.

Phillips and Rogers have described a simple modification to the medium in which the ability of *Clostridium difficile* to produce p-cresol from p-hydroxyphenyl acetic acid is used for the rapid presumptive identification by gas chromatographic detection of the p-cresol. Addition of 7% horse blood to the agar base increases the recovery of *Clostridium difficile* and produces larger colonies compared with Egg Yolk Emulsion used by George et al.

## Content concentrations

Typical Formula*	mg/litre
<b>Clostridium difficile Agar Base</b>	
Proteose peptone	40
Disodium hydrogen phosphate	5
Potassium dihydrogen phosphate	1
Magnesium sulphate	0.1
Sodium chloride	2
Fructose	6
Agar	15
Final pH 7.4 ± 0.2 @ 25°C	
<b>Clostridium difficile Selective Supplement</b>	
<a href="#">D-cycloserine</a>	250
<a href="#">Cefoxitin</a>	8
* Adjusted as required to meet performance standards	

**Table 1 - Typical Formula for *Clostridium difficile* Agar Base and *Clostridium difficile* Selective Supplement**

## METHOD

### Preparation

Suspend appropriate amount of *Clostridium difficile* Agar Base in distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C and add aseptically the contents of 1 vial of Clostridium Difficile Supplement reconstituted as directed, together with 7% (v/v) defibrinated horse blood).sheep blood may be used in place of horse blood but some strains of the organism will show a slightly reduced growth recovery. Mix well and pour into sterile Petri dishes.

### Protocol

1. Lightly inoculate the medium with the faecal sample spreading part of the original inoculum in order to obtain well separated colonies.
2. Incubate plates at 35°C for 18-24 hours in a conventional anaerobic gas jar.

3. Colonies of *Clostridium difficile* after 48 hours incubation are 4-6 mm diameter irregular, raised opaque, grey-white.

Protocol for Alcohol Shock Treatment (if required):

1. Mix equal parts of industrial methylated spirit or absolute alcohol and the faecal specimen.
2. Homogenise using a vortex mixer.
3. Leave at room temperature for 1 hour.
4. Inoculate on to Clostridium difficile Selective Agar and incubate anaerobically.

## Quality control

Positive control:

*Clostridium difficile* NCTC 11204: Good growth; grey-white coloured colonies

Negative control:

*Staphylococcus aureus* ATCC® 25923: No growth

*Escherichia coli* ATCC® 25922: No growth

## REFERENCES

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