Clostridium Difficile Moxalactam Norfloxacin (CDMN) Selective Supplement

PRODUCT INFORMATION

N008-1g - Norfloxacin, Powder, 1g

N008-5g - Norfloxacin, Powder, 5g

M031-100mg - Moxalactam Diammonium Salt, Powder, 100mg

DESCRIPTION

Clostridium difficile Agar Base with *Clostridium difficile* Moxalactam Norfloxacin (CDMN) Selective Supplement is a medium for the isolation of *Clostridium difficile*.

BACKGROUND

Cysteine (abbreviated as Cys or C) is an α-amino acid with the chemical formula HO2CCH(NH2)CH2SH. It is a non-essential amino acid, which means that it is biosynthesized in humans. Its codons are UGU and UGC. The side chain on cysteine is thiol, which is nonpolar and thus cysteine is usually classified as a hydrophobic amino acid. The thiol side chain often participates in enzymatic reactions, serving as a nucleophile. The thiol is susceptible to oxidization to give the disulfide derivative cystine, which serves an important structural role in many proteins.

Norfloxacin is a synthetic chemotherapeutic antibacterial agent. Norfloxacin is a second generation synthetic fluoroquinolone (quinolone) developed by Kyorin Seiyaku.

Moxalactam is an oxacephem antibiotic usually grouped with the cephalosporins. It has been described as a third generation cephalosporin

Mechanism of action

Norfloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell division. This mechanism can also affect mammalian cell replication.

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*.

Clostridium difficile CDMN medium is an alternative selective medium based on a formula described by Aspinall et al. for the isolation of *Clostridium difficile* from faeces. It has been found to be significantly more productive than CCFA medium. Inclusion of cysteine hydrochloride speeds the growth rate of *Clostridium difficile*. CDMN medium was reported to isolate 20% more *Clostridium difficile* strains than CCFA and the use of norfloxacin and moxalactam as selective agents reduces the number of contaminating micro-organisms by 30% when compared to CCF.

Pre-treatment of specimens with alcohol is not necessary with this medium but its use will further enhance

selectivity. See Clostridium difficile Selective Supplement for the technique.

Content concentrations

Typical Formula*	mg/litre
Clostridium difficile **Agar Base**	
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. ± 0.2 @ 25°C	
Clostridium difficile **Moxalactam Norfloxacin (CDMN) Selective Supplement**	
Cysteine hydrochloride	500
Norfloxacin	12
Moxalactam	32
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Clostridium difficile Agar Base and Clostridium difficile Moxalactam Norfloxacin (CDMN) 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. Aseptically add 2 ml of sterile distilled water and mix gently to dissolve the supplement completely. Avoid frothing. Add to 500 ml of *Clostridium difficile* Agar Base, prepared as directed and cooled to 50°C. Add 7% v/v of defibrinated horse blood. Mix well and pour into 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.

Quality control

Positive control:

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

Negative control:

Escherichia coli ATCC® 25922: No growth

Clostridium perfringens ATCC® 13124: No growth

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