MAST® Culture Media and Supplements

Technical Information Sheet

Product Code DM 254

Clostridium Difficile Medium

For the isolation of *Clostridioides difficile* from faeces.

1. Description

Clostridioides difficile and its enterotoxin are a recognised cause of pseudomembraneous (antimicrobial agent associated) colitis and of colitis and diarrhoea without pseudo-membraneous changes after antibiotic therapy. Evidence is also appearing implicating *Cl.difficile* in non-antibiotic associated colitis, post operative diarrhoea and exacerbations of chronic inflammatory disease.[1] A solid culture medium is of great value for the isolation of the organism, as the absolute count of *Cl.difficile* in faeces may be an important determinant of whether an individual develops disease, of disease type or of its severity. Often recognition of *Cl.difficile* may be obscured by the overgrowth of less exacting organisms, therefore, it is important that a selective medium is used. MAST Clostridium Difficile Medium is a modifed version of the formulation proposed by George *et al.*2 When supplemented with MAST Clostridium difficile Selectavial (SV23), and 5- 7% defibrinated horse blood, the medium then contains the correct concentrations of fructose, cycloserine, cefoxitin to act as a substitute to the egg yolk emulsion used by George *et al* [2]

MAST Clostridium Difficile Medium selectively supports the growth of *Cl.difficile* whilst inhibiting the majority of Enterobacteriaceae, *Streptococcus faecalis*, staphylococci, Gram negative, non-sporing anaerobic bacilli, and *Clostridia* spp. with the exception of *Cl.difficile*.

*Formulation may be modified to meet performance criteria

2. Technical Formula*

Formula	grams per litre
Peptone mixture	30.5
Sodium chloride	5.0
Casein hydrolysate enzymic	8.5
Fructose	6.0
Disodium hydrogen phosphate	0.5
Yeast extract	1.0
Potassium dihydrogen phosphate	0.1
Magnesium sulphate	0.12
Agar	12.0
pH approx. 7.4	

3. In Use

Clostridium Difficile Medium, supplemented with the appropriate antibiotics, can be used to culture from faecal samples, either directly or after enrichment. Addition of 5-7% horse blood to the medium may increase the recovery of *Cl.difficile* and also produce larger colonies.

Dry plates should be inoculated, spreading part of the original inoculum in order to obtain well separated colonies and incubated anaerobically at 37°C for 24-48 hours. Colonies of *Cl.difficile* will grow to 4-6mm in diameter after 48hrs incubation, appearing yellow in colour, the medium immediately surrounding the colony often becoming orange due to pigmentation. After 24 hours colonies of *Cl.difficile* can be readily distinguished from other organisms that occasionally grow on the medium.

4. Directions

1. Suspend by swirling 63.7g of powder in 1 litre or the contents of the sachet in the stated volume of distilled or deionised water.

2. Ensure the medium is evenly suspended, boil gently to dissolve the agar and autoclave at 121°C (15 p.s.i.) for 15 minutes.

3. Cool to 50-55°C and hold in a water bath at this temperature.

4. Reconstitute one Clostridium difficile Selectavial (SV23) by asceptically adding 5ml sterile water using a sterile needle and syringe. Draw the dissolved supplement up into the syringe and add to 500ml medium and mix well.

5. Supplement the medium with 5-7% defibrinated horse blood, mix well and pour culture plates of normal thickness, (15-20ml per plate). Allow to set.

6. Poured plates may be used immediately or stored at 4°C for 2 weeks in plastic bags.

5. References

1. Bolton RP, Sherriff RJ, Read AE. *Lancet* 1980; **1:** 383-384.

2. George WL, Sutter VL, Citron D, Finegold SM. *J Clin Microbiol* 1979; **9:** 214-221.

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