Technical Information

Clostridium Difficile MiVeg Agar Base

Product Code : VM1836

Application:- Clostridium Difficile MiVeg Agar Base with supplement is a selective media, used for cultivation of *Clostridium difficile* from food and certain pathological specimens.

Composition

Ingredients	Gms / Litre
MiVeg peptone No. 3	40.0
Disodium phosphate	5.0
Monopotassium phosphate	1.0
Magnesium sulphate	0.1
Sodium chloride	2.0
Fructose	6.0
Agar	15.0
Final pH (at 25°C)	7.4±0.2

** Formula adjusted, standardized to suit performance parameters.

Principle & Interpretation

Clostridium Difficile MiVeg Agar Base is prepared by using MiVeg peptone No.3 in place of Proteose peptone which is free of BSE/TSE risks. This media is the modification of Clostridium Difficile Agar Base prepared as per the formulation of George et al (1). Smith and King (2) first reported the presence of *Clostridium difficile* in human infections.
D-Cycloserine and Cefoxitin inhibits the growth of the majority of *Enterobacteriaceae* and also *Enterococcus faecalis*, *Staphylococci*, gram-negative anaerobic bacilli and *Clostridium* species other than *Clostridium difficile* which may be found abundantly in faecal samples. By adding 7% v/v horse blood the recovery of *Clostridium difficile* and its colony size get increased. For inoculation, spread the part the faecal sample on the medium to obtain isolated colonies. Incubate the plates anaerobically at 37°C for 18 - 48 hours. Greyish white, irregular, raised and opaque colonies, 4-6 mm in diameter are formed by *Clostridium difficile* after 48 hours. Gram stain morphology of *Clostridium difficile* is seen different in colonies taken from this medium due to the presence of antibiotics. It does'nt contain neutral red indicator as it is formulated for use with sheep or horse blood (1). Other tests like detection of specific cytotoxin and clinical observations should be done. Subculturing may be done on Blood Agar Base MiVeg (VM1073) to observe characteristic morphology.

Methodology

Suspend 34.5 grams of powder media in 500 ml distilled water. Mix thoroughly. Bring gently to boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow to cool to 50°C. Aseptically add Clostridium Difficile Supplement (MS2010) together with 7% (v/v) defibrinated Horse blood or Sheep blood. Mix well and pour into sterile petri plates.

Quality Control

Physical Appearance

Yellow coloured, may have slightly greenish tinge,homogeneous, free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Basal medium forms light amber coloured, slightly opalescent gel. With the addition of 7% v/v defibrinated horse blood, cherry red coloured opaque gel forms in petri plates.

Reaction

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

pH range

7.2-7.6

Cultural Response/Characteristics

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours under anaerobic condition with added Clostridium Difficile Supplement (MS2010).

Organisms (ATCC)	Inoculum (CFU)	Growth	Colour of colony	Recovery
Clostridium difficile (11204)	10^2-10^3	good-luxuriant	greyish-white	>50%
Escherichia coli (25922)	10^2-10^3	inhibited	-	0%
Shigella flexneri (12022)	10^2-10^3	inhibited	-	0%
Staphylococcus aureus (25923)	10^2-10^3	inhibited	-	0%

Key : * = with the addition of selective supplements.

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. George W.L., Sutter V.L., Citron D. and Finegold S.M., 1976, J.Clin. Microbiol., 9:214.
2. Smith L.D.S. and King E.O., 1962, J.Bact., 84:65.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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