PRODUCT SPECIFICATION SHEET

Clostridium Difficile Agar Base (DM062)

Intended Use
Clostridium Difficile Agar Base (DM062) is recommended for selective isolation of *Clostridium difficile* from faeces.

Product Summary and Explanation
Clostridium difficile is a pathogenic *Clostridium* affecting the bowel. The spectrum of disease caused by *Clostridium difficile* ranges from pseudomembranous colitis (PMC) through antibiotic associated colitis (AAC). It is now one of the most commonly detected enteric pathogens, and an important cause of nosocomial infections in hospitals and nursing homes.[1] It also includes chronic inflammatory bowel diseases, post-operative diarrhoea and non-antibiotic associated diarrhoea.[2] The organism has been isolated from diverse natural habitats, including soil, hay, sand, dung from various large mammals, and from dog, cat, rodent and human feces. Smith and King[3] first reported the presence of *C. difficile* in human infections. In 1979, George et al. developed a medium called CCFA (cycloserine-cefoxitin-fructose agar), based on the Egg Yolk Agar formula of McClung and Toabe with fructose replacing glucose.[4] Clostridium Difficile Agar is a modification of the original CCFA formulation.[1] 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.[4] Clostridium Difficile Agar Base is used for the primary isolation of *C. difficile* from faecal specimens.

Principles of the Procedure
Clostridium Difficile Agar Base contains proteose peptone which provides nitrogen, vitamins, and amino acids in Clostridium Difficile Agar. Fructose is the fermentable carbohydrate used to enhance recovery and growth of *C. difficile*. The Phosphates are buffering agents in this medium. Magnesium Sulfate is a source of inorganic ions to stimulate growth. Sodium Chloride maintains the osmotic balance of the medium. 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.

Formula / Liter

Ingredients	Gms / Liter
Proteose peptone	40.00
Disodium phosphate	5.00
Monopotassium phosphate	1.00
Magnesium sulphate	0.10
Sodium chloride	2.00
Fructose	6.00
Agar	15.00
Final pH: 7.4 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 34.55 grams of the medium in 500ml of distilled water.
2. Heat gently to boiling to dissolve the medium completely.

3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 50°C. Aseptically add rehydrated contents of 1 vial of Clostridium Difficile Supplement (MS011) together with 7% (v/v) defibrinated Horse blood or Sheep blood.
5. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
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 of 6.91% solution	pH 7.4 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed under anaerobic condition with added Clostridium Difficile Supplement(MS011) and 7% v/v defibrinated horse blood, after an incubation at 35-37°C for 48 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Colour of Colony
1.	*Clostridium difficile ATCC 11204*	50-100	good-luxuriant	>=50%	greyish-white
2.	*Shigella flexneri ATCC 12022*	>=10^3	inhibited	0%	
3.	*Escherichia coli ATCC 25922*	>=10^3	inhibited	0%	
4.	*Staphylococcus aureus ATCC 25923*	>=10^3	inhibited	0%	

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. 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.
2. Refer to appropriate references for a complete discussion on the isolation and identification of *C. difficile* and other anaerobic bacteria.[1,5]

Results

1. *C. difficile* forms grayish white, irregular, raised and opaque colonies, 4-6 mm in diameter, after 48 hours.
2. Typical gram stain morphology of *C. difficile* may not be seen in colonies taken from this medium due to the presence of antibiotics.
3. Subculture on Blood Agar to obtain characteristic morphology.
4. *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.
5. Refer to appropriate references and standard test procedures for interpretation of results.

Results

Examine plates for fungal colonies exhibiting typical color and morphology. Biochemical tests and serological procedures should be performed to confirm findings.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Clostridium Difficile Agar does not contain Neutral Red indicator because it is designed for use with horse blood.
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Clostridium Difficile Agar Base
Product Code : DM062
Available Pack sizes : 500gm

References

1. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 14th Ed., Churchill Livingstone.
3. Smith L. D. S. and King E. O., 1962, J. Bacteriol., 84:65.
4. George W. L., Sutter V. L., Citron D., and Finegold S. M., 1979, J.Clin. Microbiol., 9:214.
5. Isenberg, H. D. (ed.). 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

Further Information

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