PRODUCT SPECIFICATION SHEET

Clostridium Brazier Agar Base (DM1063)

Intended Use

Clostridium Brazier Agar Base (DM1063) is recommended for selective isolation and differentiation of *Clostridium* difficle.

Product Summary and Explanation

Clostridium difficile is a pathogenic *Clostridium* affecting the bowel leading to diarrhea and more serious intestinal conditions such as colitis. The spectrum of disease caused by *Clostridium difficile* ranges from pseudomembranous colitis (PMC) through antibiotic associated colitis (AAC). It also includes chronic inflammatory bowel diseases, post-operative diarrhoea and non-antibiotic associated diarrhoea.[1] The presence of *C. difficile* in human infections was first reposted by Smith and King.[2] Clostridium Brazier Agar Base was developed by Jon Brazier[3] based on similar work carried out by Ken Phillips and Paul Levett. Anaerobe Reference Unit is using this medium for isolating *C. difficile* alon with many pathological labs.

Principles of the Procedure

Clostridium Brazier Agar Base contains peptone special which provides nitrogen and amino acids required for growth. Sodium chloride helps to maintain the osmotic balance of the medium. Cholic acid present in the medium promotes spore germination following shock treatment, and p-hydroxyphenylacetic acid to enhance production of p-cresol, a distinctive metabolite of *C. difficile*. The selective agents in Clostridium difficile supplement (MS011), D-cycloserine and cefoxitin used in this medium inhibits the growth of the 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 samples. The Egg Yolk Emulsion (MS038) added to the medium helps to differentiate *C. difficile* from lecithinase positive Clostridia. Addition of lysed horse blood to the base enhances recognition of colony fluorescence when cultures are examined using UV light.

Formula / Liter

Ingredients	Gms / Liter
Peptone special	23.00
Sodium chloride	5.00
Starch, soluble	1.00
Sodium bicarbonate	0.40
Dextrose	1.00
Sodium pyruvate	1.00
Cysteine HCL	0.50
Haemin	0.01
Vitamin K	0.001
L-Arginine	1.00
Sodium pyrophosphate	0.25
Sodium succinate	0.50
Cholic acid	1.00
p-Hydroxyphenylacetic acid	1.00
Agar	12.00
Final pH : 7.0 ± 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 47.66 grams of the medium in one litre distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4. Cool to 45-50°C. Aseptically add rehydrated contents of 2 vials of Clostridium Difficile Supplement (MS011), 40 ml of Egg Yolk Emulsion (MS038) together with 10 ml lysed horse 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 of Egg yolk emulsion (MS038) and 10 ml lysed horse blood: Tan coloured opaque gel forms in Petri plates
Reaction of 4.76% Solution	pH : 7.0 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.2% Agar gel

Expected Cultural Response: Cultural characteristics observed under anaerobic condition with added Clostridium Difficile Supplement (MS011), Egg yolk Emulsion (MS038) and 10 ml of lysed 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	Lecithinase activity
1.	*Clostridium difficile ATCC 11204*	50-100	good-luxuriant	>=50 %	greyish-white, opaque flat colonies	negative
2.	*Escherichia coli ATCC 25922*	>=10^3	inhibited	0 %	--	--

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

Test Procedure

Refer to appropriate references for standard test procedures.

Results

Typical characteristics of *C. difficile* appear on this medium after 24 hours on anaerobic incubation at 35-37°C. *C. difficile* appears as grey, opaque, flat raised colonies generally circular but may tend to elongate, which on further incubation upto 48 hours may result in lighter grey or may impart white centre to the medium and form opaque colonies, 4-6 mm in diameter. Refer to appropriate references for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 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. Typical Gram stain morphology of *C. difficile* may not be seen in colonies taken from this medium due to the presence of antibiotics.
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 Brazier Agar Base
Product Code : DM1063
Available Pack sizes : 500gm

References

1. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 14th Ed., Churchill Livingstone.
2. Smith L. D. S. and King E. O., 1962, J. Bacteriol., 84:65.
3. Brazier J S (1993) Role of the Laboratory in Investigations of Clostridium difficile Diarrhoea. Clinical Infectious Diseases 16 (4) S228-33.

Further Information

For further information please contact your local MICROMASTER Representative.

MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall' , Thane (W) – 400607. M.S. INDIA.
Ph: +91-9320126789/9833630009/9819991103
Email: sales@micromasterlab.com

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