Clostridium Brazier Agar Base

M1803

Intended Use:

Recommended as selective media for isolation and differentiation of *Clostridium difficile* with added supplements.

Composition**

Ingredients	Gms / Litre
Peptone special	23.000
Sodium chloride	5.000
Starch, soluble	1.000
Sodium bicarbonate	0.400
Dextrose (Glucose)	1.000
Sodium pyruvate	1.000
L-Cysteine hydrochloride	0.500
Hemin	0.010
Vitamin K	0.001
L-Arginine	1.000
Sodium pyrophosphate	0.250
Sodium succinate	0.500
Cholic acid	1.000
p-Hydroxyphenylacetic acid	1.000
Agar	12.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 47.66 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 2 vials of Clostridium Difficile Supplement (FD010), 40 ml of Egg Yolk Emulsion (FD045) together with 10 ml lysed horse blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The spectrum of disease caused by *Clostridium difficile* (a pathogenic *Clostridium* affecting the bowel) 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 (2). Smith and King (6) first reported the presence of *C. difficile* in human infections.

This medium was developed by Jon Brazier (1) based on similar work carried out by Ken Phillips and Paul Levett. Many pathological labs including Anaerobe Reference Unit are using this medium for isolating *C. difficile*. Typical characteristics of *C. difficile* appears 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. Typical Gram stain morphology of *C. difficile* may not be seen in colonies taken from this medium due to the presence of antibiotics. The selective agents in Clostridium difficile supplement (FD010), 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 (FD045) 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. 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*.

Type of specimen
Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions
Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Gram stain morphology of *C. difficile* may not be seen in colonies taken from this medium due to the presence of antibiotics.
2. Some isolates may show poor growth due to nutritional variations.

Quality Control
Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.2% Agar gel

Colour and Clarity of prepared medium
Basal medium: Light amber coloured clear to slightly opalescent gel. After addition of Egg yolk emulsion (FD045) and 10 ml lysed horse blood: Tan coloured opaque gel forms in Petri plates.

Reaction
Reaction of 4.76% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed under anaerobic condition with added Clostridium Difficile Supplement(FD010),Egg yolk Emulsion (FD045) and 10 ml of lysed horse blood, after an incubation at 35-37°C for 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Lecithinase activity
Clostridium difficile ATCC 11204	50-100	good-luxuriant	>=50%	greyish-white, opaque flat colonies	negative
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%		

Key :(*) Corresponding WDCM numbers.

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Brazier J S (1993) Role of the Laboratory in Investigations of *Clostridium difficile* Diarrhoea. Clinical Infectious Diseases 16 (4) S228-33.

2. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 14th Ed., Churchill Livingstone.

3. Isenberg, H.D. Clinica Microbiology Procedures Handbook 2nd Edition.

4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

6.Smith L. D. S. and King E. O., 1962, J. Bacteriol., 84:65.

Revision : 02 / 2019