TM 2042 – CLOSTRIDIUM BRAZIER AGAR BASE

INTENDED USE

For isolation and differentiation of *Clostridium difficile* with added supplements.

PRODUCT SUMMARY AND EXPLANATION

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 Smith and King first reported the presence of *C. difficile* in human infections.

This medium was developed by Jon Brazier 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, 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 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 pcresol, a distinctive metabolite of C. difficile.

COMPOSITION

Ingredients	Gms / Ltr
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

PRINCIPLE

The medium contains peptone, starch and dextrose provides nutrients to the medium. Sodium chloride helps in the osmotic balance in the medium. Agar present helps in solidifying the medium.

INSTRUCTION FOR USE

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

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.

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

pH (at 25°C) : 7.0±0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony	Lecithinase activity	Incubation Temperature	Incubation Period
Clostridium difficile	11204	50-100	Good-luxuriant	>=50%	Greyish-white, opaque flat colonies	Negative	35-37°C	48 Hours
Escherichia coli	25922	>=10^3	Inhibited	0%	-	-	35-37°C	48 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

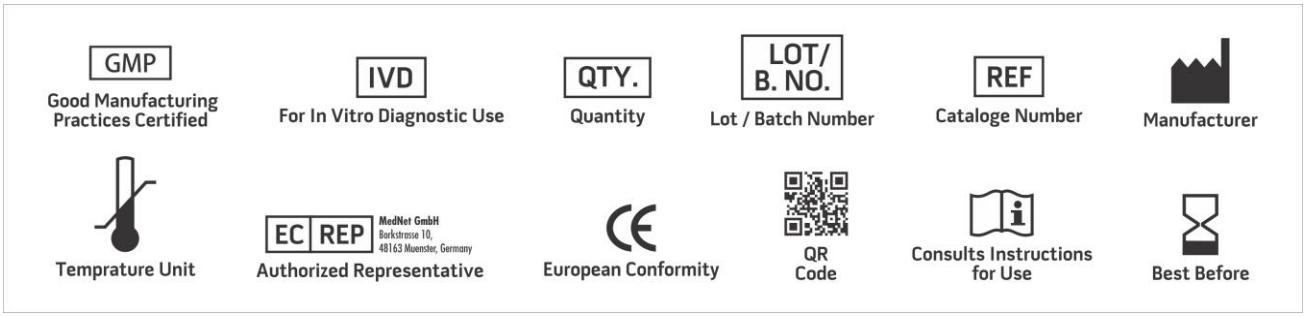
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

Revision: 08 Nov., 2019