CM 20486 – CLOSTRIDIUM DIFFICILE AGAR BASE

INTENDED USE

For isolation of Clostridium dificille from food and pathological specimens.

PRODUCT SUMMARY AND EXPLANATION

Thespectrum 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. George et al recommended the use of a fructose-containing medium with egg yolk for the isolation of C.difficile from faecal specimens. 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. Clostridium Difficile Agar Base is used for the primary isolation of C.difficilefrom faecal specimens. The medium composition is designed so as to obtain luxuriant growth of C.difficile. 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.
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. C. difficile forms grayish white, irregular, raised and opaque colonies, 4-6 mm in diameter, after 48 hours. Typical gram stain morphology of C. difficile may not be seen in colonies taken from this medium due to the presence of antibiotics. Subculture on Blood Agar to obtain characteristic morphology. 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.

COMPOSITION

Ingredients	Gms / Ltr
Proteose peptone	40.000
Disodium hydrogen phosphate	5.000
Potassium dihydrogen phosphate	1.000
Magnesium sulphate	0.100
Sodium chloride	2.000
Fructose	6.000
Agar	15.000

PRINCIPLE

Themediumcontains peptone and fructose 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 34.55 grams in 500 ml purified / distilled water.
Heat gently 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 1 vial of Clostridium Difficile Supplement together with 7% (v/v) defibrinated Horse blood or Sheep 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 7% v/v defibrinated horse blood: Cherry red coloured, opaque gel forms in Petri plates.

pH (at 25°C) : 7.4±0.2

INTERPRETATION

Cultural characteristics observed after incubation under anaerobic condition with added Clostridium Difficile Supplement and 7% v/ v defibrinated horse blood.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony	Incubation Temperature	Incubation Period
Clostridium difficile	11204	50-100	Good-luxuriant	>=50%	Greyish-white	35-37°C	48 Hours
Shigella flexneri	12022	>=10^3	Inhibited	0%	-	35-37°C	48 Hours
Escherichia coli	25922	>=10^3	Inhibited	0%	-	35-37°C	48 Hours
Staphylococcus aureus	25923	>=10^3	Inhibited	0%	-	35-37°C	48 Hours

PACKAGING:

Inpacksizeof500 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.

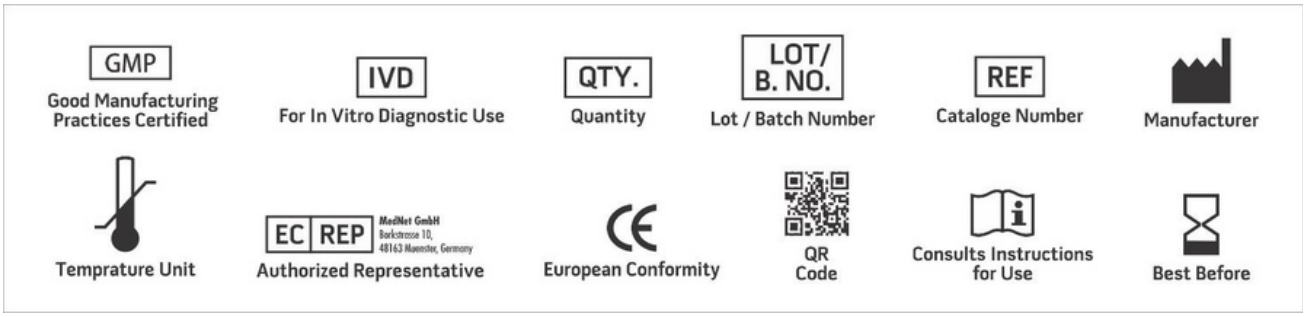
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

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

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. George W. L., Sutter V. L., Citron D., and Finegold S. M., 1979, J.Clin. Microbiol., 9:214.



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

*For LabUse Only