

## 17145 *Clostridium difficile* Agar (Base) NutriSelect® Plus

Used with supplement for cultivation of *Clostridium difficile* from food and certain pathological specimens.

### Composition:

Ingredients	Grams/Litre
Proteose peptone	40.0
Disodium phosphate	5.0
Monopotassium phosphate	1.0
Magnesium sulfate	0.1
Sodium chloride	2.0
Fructose	6.0
Agar	15.0

Final pH 7.4 +/- 0.2 at 25°C

Store dehydrated powder between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Appearance(color): Faint Yellow to light yellow & faint beige to light beige & faint brown to light brown, free flowing powder  
 Gelling: Firm, comparable with 1.5% Agar gel.  
 Color and Clarity: 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.

### Directions:

Suspend 34.55 g in 500 ml of distilled water. Heat gently to boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add rehydrated contents of 1 vial of *Clostridium Difficile* Supplement (Cat. No. 17122) together with 7% (v/v) defibrinated Horse blood or Sheep 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 (3) first reported the presence of *C.difficile* in human infections. George *et al* (1) in a study of selective media for the routine isolation of *Clostridium difficile* from faecal specimens found this medium was inhibitory compared with growth on blood agar. They recommended the use of a fructose containing nutrient medium plus egg yolk, with D-cycloserine and cefoxitin as selective agents for the isolation of *Clostridium difficile*.

This medium does not contain neutral red indicator, as in the original formulation, as it is recommended for use with sheep or horse blood (1). *Clostridium Difficile* Agar Base is used for the primary isolation of *C.difficile* from faecal specimens. The medium composition is designed so as to obtain luxuriant growth of *C.difficile*. The selective agents D-cycloserine (500µg/ml) and cefoxitin (16µg/ml) inhibit growth of the majority of *Enterobacteriaceae*, as well as *Streptococcus faecalis*, *staphylococci*, Gram-negative non-sporing anaerobic bacilli and *Clostridia* species. (except *Clostridium difficile*) which may be found in large numbers in faecal samples. Addition of 7%



horse blood to the agar base increases the recovery of *Clostridium difficile* and produces larger colonies compared with Egg Yolk Emulsion used by George *et al*(1).

Spread a part of the faecal sample on the medium in order to obtain well separated 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.

Cultural characteristics observed with added Clostridium Difficile Supplement and 7% v/v defibrinated horse blood, after an incubation of 48 hours at 35-37°C under anaerobic conditions

Organisms (ATCC/WDCM)	Inoculum (CFU)	Growth	Recovery	Color of colony
<i>Clostridium difficile</i> (11204/-)	50-100	++/+++	≥50%	greyish white
<i>Shigella flexneri</i> (12022/-)	>=10 <sup>4</sup>	-	0%	-
<i>Escherichia coli</i> (25922/00013)	>=10 <sup>4</sup>	-	0%	-
<i>Staphylococcus aureus</i> (25923/-)	>=10 <sup>4</sup>	-	0%	-

#### References:

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

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

