Clostridium Difficile Agar Base

Cat. 1447

For the isolation of Clostridium difficile.

Practical information

Aplications	Categories
Selective isolation	Clostridium difficile

Industry: Clinical / Food

Principles and uses

Clostridium Difficile Agar Base, when used with supplements, is a selective medium for the isolation of Clostridium difficile from food and fecal specimens.

Clostridium difficile was first isolated from meconium and infant feces in 1935 by Hall and O'Toole, who proposed the name "difficile" because it was very difficult to isolate. Keighley associated Clostridium difficile with colitis and diarrhea after antibiotic therapy following gastrointestinal operations. In 1979, George et al developed a medium called CCFA (cycloserine-cefoxitin-fructose agar), which is a bloodless medium, based on the egg yolk agar of McClung and Toabe with fructose as a glucose replacement. The combination of Clostridium Difficile Agar with the supplement is based on this medium.

The selective agents D-cycloserine, and cefoxitin inhibit the growth of most Enterobacteriaceae, as well as Enterococcus faecalis, staphylococci, Gram negative, non-sporing anaerobic bacilli, and Clostridia species (except Clostridium difficile), which may be found in large quantities in fecal samples.

Peptone proteose provides nitrogen, vitamins, minerals and amino acids essential for growth. Fructose is the fermentable carbohydrate used to enhance recovery and growth of C. difficile. Potassium dihydrogen phosphate and disodium hydrogen phosphate act as a buffer system. Magnesium sulfate is an ion required in a large variation of enzymatic reactions, including DNA replication. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Horse blood provides essential growth factors. Bacteriological agar is the solidifying agent.

Formula in g/L

Bacteriological agar	15	Magnesium sulfate	0,1
Potassium dihydrogenphosphate	1	Sodium chloride	2
Di-sodium hydrogen phosphate	5	Fructose	6
Proteose peptone	40		

Preparation

Suspend 34,5 grams of the medium in 500 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. Cool to 45-50 ºC and aseptically add one vial of Clostridium Difficile Supplement (Cat. 6061) and 7% sterile defibrinated horse blood. Homogenize gently and dispense into Petri dishes. Be careful to avoid
bubble formation when adding the blood and rotate the flask or bottle slowly to create a homogeneous solution.

Sheep blood may be used instead of horse blood, but some strains of the organisms will show a slightly reduced growth recovery.

Instructions for use

- In a Petri dish, add 12-15 ml of molten agar and let it solidify.
- Lightly inoculate the medium with the faecal sample spreading 10 µl of the original inoculum in order to obtain well separated colonies.
- Incubate plates at 35-37 ºC for 18-48 hours in a conventional anaerobic jar.
- Colonies of Clostridium difficile after 48 hours incubation are 4-6mm diameter irregular, raised opaque, grey-white.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Brown (when blood is added)	7,4±0,2

Microbiological test

Incubation conditions: (35-37 ºC / 18-48 h).

Microorganisms	Specification
Escherichia coli ATCC 25922	Total inhibition
Staphylococcus aureus ATCC 25923	Total inhibition
Clostridium difficile ATCC 9689	Good growth

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

George W. L., Sutter V.L., Goldstein E.C.J., Ludwig S.L and Finegold S.M (1978) Lancet. i. 802-803
Hall I. and O'Toole E. (1935) am. J. Dis. Child. 49. 390.
Keighley M.R.B, Burdon D.W., Alexander Williams J. et al (1978) Lancet ii. 1165-1167.