Condalab

Reference: 6061 **Technical Data Sheet**

Product: **CLOSTRIDIUM DIFFICILE SUPPLEMENT**

Specification

Sterile selective supplement used for the isolation of *Clostridium difficile.*

Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze-dried vials Vial with: ± 0.5 ml	22±0.25 x 55±0.5 mm glass vials, tag labelled, White plastic cap - 10 vials per box.	36 months	2-8 ºC

Composition

Composition (g/vial):
D-Cycloserine.....0,125
Cefoxitin..... 0,004

Note: Each vial is sufficient to supplement for 500 ml of medium Base Agar Clostridium Difficile

Reconstitute the original freeze-dried vial by adding
Sterile Distilled Water.....5 ml

Description /Technique

Description:
Clostridium Difficile Agar Base (Cat. 1447), 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.

Technique:
Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and add aseptically to 500 ml of Clostridium Difficile Agar Base (Cat. 1447) autoclaved and cooled to 50 ºC, together with 7% of defibrinated horse blood. Mix well and distribute into Petri dishes.

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

Physical/Chemical control

Color : Yellowish pH: at 25ºC

Microbiological control

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely

Add 1 vial to 500 ml of medium base. DO NOT HEAT once supplemented.

Inoculate: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/ 10^4-10^6 (selectivity).

5% CO2 atmosphere. Incubation at 35-37 ºC during 24-48 h.

Microorganism	Growth
Stph. aureus ATCC® 25923, WDCM 00034	Inhibited
Escherichia coli ATCC® 25922, WDCM 00013	Inhibited
Clostridium difficile ATCC® 43255	Good

Sterility Control

Add 5ml of the sample to 100 ml of TSB and to 100 ml Thioglycollate.
Incubation 48 hours at 30-35 ºC and 48 hours at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

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.