# CLOSTRIDUM DIFFICILE AGAR

- For in vitro use only - Catalogue No. PC62

Our Clostridium Difficile Agar is a selective medium used for the isolation of *C. difficile* from clinical specimens.

*Clostridium difficile* was first isolated in 1935 by Hall and O'Toole who proposed the name 'difficile' due their difficulty in isolating it. Numerous selective formulations for *C. difficile* have been published one of these being by George et al.; they recommended the use of a fructose containing medium along with egg yolk plus the selective agents cycloserine and cefoxitin. Our formulation is based on their work, except that the concentrations of the two antibiotics have been reduced due to that some strains of *C. difficile* were reported to be inhibited on the original medium; the egg yolk has also been replaced with 7% horse blood as the blood has reported to increase recovery and produce larger colonies than egg yolk.

This formulation does not contain the color indicator neutral red as proposed by George et al. because of the presence of horse blood, therefore no color reaction will occur on this medium, however the fluorescent reaction will still occur on this medium.

**Formula per Litre of Medium**

| | |
|---|---|
| Proteose Peptone | 40.0 g |
| Sodium Phosphate Dibasic | 5.0 g |
| Potassium Phosphate Monobasic | 1.0 g |
| Magnesium Sulfate | 0.1 g |
| Sodium Chloride | 2.0 g |
| Fructose | 6.0 g |
| Agar | 15.0 g |
| D-Cycloserine | 250 mg |
| Cefoxitin | 8 mg |
| Defibrinated Horse Blood | 70.0 mL |

pH 7.4 ± 0.2

**Recommended Procedure**

1. Allow medium to reach room temperature.
2. Using an inoculum from the specimen, perform a four-quadrant streak to obtain well-isolated colonies. If the specimen is contained on a swab, roll the swab several times over a small area near the edge of the plate and streak the plate for isolation with a sterile loop starting where the swab was inoculated.
3. Incubate anaerobically at 35°C.
4. Examine plates after 48 hours. Re-incubate plates an additional 24 hours if required.

**Interpretation of Results**

*Clostridium difficile* colonies will typically appear as grey-white, raised, opaque, 4 to 6-mm in diameter. The colonies will fluoresce yellow under UV light. Most other organisms are inhibited on this medium.

Additional biochemical and/or serological tests should be performed on isolated colonies from pure culture in order to complete identification.

- *C. difficile may not appear normal microscopically due to the presence of the antibiotics affecting their cellular morphology. Subculture onto a non-selective medium for staining purposes*

- *Alcohol shock treatment of fecal specimens may also be used on this medium to improve recovery of C. difficile*

## Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

| Organism | Expected Result |
|---|---|
| *Clostridium difficile* ATCC 9689 | Growth, grey, opaque colonies |
| *Staphylococcus aureus* ATCC 25923 | Partial to complete inhibition |
| *Escherichia coli* ATCC 25922 | Partial to complete inhibition |

## Storage and Shelf Life

Our Clostridium Difficile Agar should be stored away from direct light at 4 to 8°C with the medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 8 weeks from the date of manufacture.

## References


1. Hall I, O'Toole E. Am J Dis Child 1935; 49:390.
2. George WL, Sutter VL, Citron D, Finegold SM. Selective and differential medium for isolation of *Clostridium difficile*. J Clin Micobiol 1979; 9:214-9.
3. Levett. J Clin Pathol 1985; 38:233-4.
4. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
5. Clabots CR, Gerding SJ, Olson MM, Peterson LR, Gerding DN. Detection of asymptomatic *Clostridium difficile* carriage by an alcohol shock procedure. J Clin Microbiol 1989; 27: 2386-7.
6. Isenberg HD, Ed. Clinical microbiology procedures handbook. Washington, DC: ASM, 1992.
7. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 10th ed. St. Louis: Mosby, 1998.



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