BD™ Clostridium Difficile Agar with 7% Sheep Blood

INTENDED USE

BD Clostridium Difficile Agar with 7% Sheep Blood is a selective medium for the primary isolation of *Clostridium difficile* from fecal and other specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Clostridium difficile is recognized as the most common cause of antibiotic-associated colitis and pseudomembraneous colitis (PMC).[1] A number of procedures have been developed for the isolation of C. difficile.[2-4] In 1979, George et al. developed a medium called CCFA (cycloserine-cefoxitin-fructose agar), which is a bloodfree medium, based on the Egg Yolk Agar formula of McClung and Toabe with fructose replacing the glucose.[5] **BD Clostridium Difficile Agar with 7% Sheep Blood** is a modification of the original CCFA formulation. The peptone concentration has been reduced to a level comparable to other media used for *C. difficile*, and the cycloserine and cefoxitin concentrations have been reduced since the original formulation was too inhibitory.[6] The egg yolk has been omitted since *C. difficile* is lecithinase and lipase negative and the medium inhibits most other clostridia. Sheep blood provides nutrients and allows good sporulation and detection of a greenish fluorescence seen under long-wave UV. [7,5] The peptone and fructose supply the necessary nitrogen and carbon sources. Phosphates maintain the pH. Cycloserin and cefoxitin are selective agents to suppress accompanying bacteria.

REAGENTS

BD Clostridium Difficile Agar with 7% Sheep Blood

Formula* Per Liter Purified Water

Peptic Digest of Animal Tissue	32.0 g
Fructose	6.0
Monopotassium Phosphate	1.0
Disodium Phosphate	5.0
Sodium Chloride	2.0
Magnesium Sulfate	0.1
Cycloserine	0.5
Cefoxitin	0.016
Agar	20.0
Sheep Blood, defibrinated	7%

pH 7,2 ± 0,3

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate anaerobically for 48 to 72 hours at 35 to 37° C.

Strains	**Growth Results**
Clostridium difficile ATCC™ 9689	Growth good to excellent; gray colonies with umbonate edges, no hemolysis
Clostridium perfringens ATCC 13124	Inhibition partial to complete, beta-hemolysis
Bacteroides fragilis ATCC 25285	Inhibition complete
Escherichia coli ATCC 25922	Inhibition complete
Proteus mirabilis ATCC 12453	Inhibition complete
Staphylococcus aureus ATCC 25923	Inhibition complete
Uninoculated	Red (blood color)

PROCEDURE

Materials Provided

BD Clostridium Difficile Agar with 7% Sheep Blood (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Collection and Specimen Types

This medium is used for the isolation of *C. difficile* from fecal specimens. Freshly passed stool specimens (ideally 10 to 20 ml of watery stool) of patients suspected to suffer from pseudomembraneous colitis (PMC) should be used. Results of testing solid, formed stools are not likely to contribute to the diagnosis of PMC. Especially hospitalized patients developing diarrhea during or shortly after antimicrobial therapy should be tested for *C. difficile* infection and PMC. *Clostridium difficile* is very sensitive to oxygen. Therefore, stool specimens must be fresh and should be processed within 2 hours if anaerobic transport media are not used (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). If specimens are placed into anaerobic transport medium vials, longer transport time is acceptable, but not longer than 24 hours. Do not freeze the specimens! [7,8]

Test Procedure

As soon as possible after receipt in the laboratory, inoculate the specimen onto a **BD Clostridium Difficile Agar with 7% Sheep Blood** plate and streak for isolation. As some strains of *C. difficile* may not grow well due to the selective properties of the medium, it is advisable to include a nonselective medium such as **BD Schaedler Agar with Vitamin K1 and 5% Sheep Blood** or **BD CDC Anaerobe Agar with 5% sheep blood**. Since some facultative anaerobic organisms potentially produce reactions similar to *C. difficile*, it is recommended that an aerobically incubated blood agar plate be included to confirm that the isolate is an obligate anaerobe.

Anaerobic media should be reduced prior to inoculation by placing under anaerobic conditions for 6 to 24 h prior to use. An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak™** anaerobic systems.

Incubate immediately under anaerobic conditions at 35 to 37°C for at least 48 h. Regardless of anaerobic system used, it is important to include an indicator of anaerobiosis such as the **BD GasPak** disposable anaerobic indicator.

Results

After 48 to 72 h incubation, *Clostridium difficile* will appear as gray-white colonies with a ground glass-like appearance and a slightly filamentous edge, but with no signs of beta-hemolysis. Well-grown cultures have a characteristic “horse-stable-like” odour, caused by accumulation of p-cresol. Examine growth with a long-wave ultraviolet light for greenish fluorescence. This should be done

within one hour after removal from the anaerobic atmosphere. After exposure to air, colonies may become rapidly nonviable which is usually accompanied by a loss of fluorescence. Further tests are necessary for the final identification of the isolates.[7-13]

Interpretation of the Results
Since the presence of *Clostridium difficile* in the culture without detecting toxin A and B of the organism is not indicative of antibiotic-associated colitis or pseudomembraneous colitis, appropriate immunological tests must be performed from the stool specimen. Also, results should be interpreted along with clinical observations.[14,15]

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

This prepared plated medium is one of the standard formulations for primary isolation of *Clostridium difficile*. Some diagnostic tests may be performed with growth from the primary plating medium. For identification, the organism must be in pure culture. Complete identification may be performed using Gram reaction, cellular morphology, sensitivity to oxygen, biochemical reactions, susceptibility to antimicrobial agents and gas liquid chromatographic analysis of metabolic products.
Other species of clostridia, e.g., *C. ramosum*, may grow and produce fluorescence on this medium. Also, the isolation of *Clostridium difficile* should not be relied upon for etiologic diagnosis of pseudomembraneous colitis (see also **Interpretation of the Results**).[14,15]
Since there is no such entity as a perfect medium, some strains of *C. difficile* may be encountered that will grow poorly on this medium; the nature of the specimens and the physiologic state of the organisms can influence recovery of desired species, as well as modify the effects of inhibitory characteristics of this medium.
Clostridium difficile is very sensitive to oxygen. Specimens and cultures exposed to air for a longer time will rapidly lose viability.[8]

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PACKAGING/AVAILABILITY

BD Clostridium Difficile Agar with 7% Sheep Blood
Cat. No. 254406 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

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