

QUALITY CONTROL PROCEDURES

I INTRODUCTION

Clostridium difficile Selective Agar (CDSA) is recommended as a selective and differential medium for the primary isolation of *Clostridium difficile* from fecal specimens.

II PERFORMANCE TEST PROCEDURE

1. Reduce plates at room temperature for 6 – 24 h prior to use in a **BD GasPak™ EZ** anaerobic system.
2. Inoculate representative samples with the cultures listed below.
 - a. For obligate anaerobes, streak inoculate with 1 µL (0.001 mL) from a 48 – 72 h culture of Chopped Meat Broth that has been incubated for 2 days at 35 – 37°C.
 - b. For *E. coli* and *P. mirabilis* streak inoculate 1 µL (0.001 mL) from a 4 – 5 h culture of **Trypticase™** Soy Broth diluted to yield 10⁶ – 10⁷ CFU/mL.
 - c. Incubate plates at 36 ± 1°C in an anaerobic atmosphere.
 - d. Include plates of a previously tested lot of **Trypticase** Soy Agar with 5% Sheep Blood as controls for inhibited strains.
3. Examine plates after 48 – 72 h for growth, colony color, fluorescence and selectivity.
4. Expected Results

Organisms	ATCC™	Recovery	Colony Color	Fluorescence
* <i>Clostridium difficile</i>	9689	Fair to heavy growth	Pale yellow to bright yellow	Yes [†]
<i>Clostridium difficile</i>	51695	Fair to heavy growth	Pale yellow to bright yellow	Yes [†]
* <i>Clostridium perfringens</i>	13124	Complete inhibition	N/A	N/A
* <i>Escherichia coli</i>	25922	Complete inhibition	N/A	N/A
* <i>Proteus mirabilis</i>	12453	Complete inhibition	N/A	N/A

*Recommended organism strain for User Quality Control.

[†]Examine colonies on the CDSA medium with a long-wave (365 nm) UV lamp. Colonies of *Clostridium difficile* should produce yellow fluorescence under UV light for up to one hour after removal from the anaerobic atmosphere.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.2 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Clostridium difficile Selective Agar (CDSA) is recommended as a selective and differential medium for the primary isolation of *Clostridium difficile* from fecal specimens.

V SUMMARY AND EXPLANATION

Clostridium difficile is recognized as the most common cause of antibiotic-associated colitis and pseudomembranous 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 based on the Egg Yolk Agar formula of McClung and Toabe with fructose replacing the glucose.⁵ It was subsequently reported that the concentration of cycloserine and cefoxitin in the original CCFA formula was inhibitory to *C. difficile*.⁶ Several modifications of CCFA and alternate formulations have been described for the culture of *C. difficile*. These include adding horse serum, sodium taurocholate, and media with mannitol replacing fructose.⁷

CDSA is a selective and differential medium developed by BD. It permits superior recovery of *C. difficile*, with equivalent to better inhibition of normal flora when compared to the BD modified formulation of CCFA (**BBL™ Clostridium difficile** Agar). As growth of *C. difficile* occurs, the pH of the medium is raised causing the neutral red indicator to turn yellow.

VI PRINCIPLES OF THE PROCEDURE

CDSA employs a peptone base with 0.6% mannitol. Ingredients have been optimized to improve recovery and colony size of *C. difficile*. Amino acids present in the agar base are utilized by *C. difficile* causing an increase in pH. The colony and surrounding medium change color from rose to yellow as the pH rises. Mannitol is utilized by fewer *Clostridium* species than fructose and improves the recovery of *C. difficile*. Cefoxitin and cycloserine are incorporated to inhibit normal fecal flora. These antibiotics have a broad range of antimicrobial activity against aerobic, anaerobic and facultatively anaerobic gram-positive and gram-negative bacteria while permitting recovery of *C. difficile*. *C. difficile* colonies produce yellow fluorescence when viewed with long-wave UV light.

VII REAGENTS

Clostridium difficile Selective Agar (CDSA)

Peptic Digest of Animal Tissue	32.0 g	Magnesium Sulfate.....	0.1 g
Mannitol	6.0 g	Agar.....	20.0 g
Monopotassium Phosphate	1.0 g	Neutral Red	0.03 g
Disodium Phosphate	5.0 g	Cycloserine	0.25 g
Sodium Chloride.....	2.0 g	Cefoxitin	0.016 g
Growth Factors	3.3 g		

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over the off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Storage Instructions: On receipt, store plates in the dark at 2 – 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 – 8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

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

VIII SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.^{8,9}

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"¹⁰⁻¹³ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

IX PROCEDURE

Material Provided: *Clostridium difficile* Selective Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

As soon as possible after receipt in the laboratory, inoculate the specimen onto a reduced CDSA 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 CDC Anaerobe Blood Agar.

Media should be reduced prior to inoculation by placing under anaerobic conditions for 6 – 24 h prior to use.¹⁴ An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BBL™ GasPak™ EZ** anaerobic systems.

Incubate immediately under anaerobic conditions or place in a holding jar flushed with oxygen-free gas(es) until sufficient plates are accumulated (but no longer than 3 h).¹⁵ Incubation should be at 35 ± 2°C for at least 48 h. Regardless of anaerobic system used, it is important to include an indicator of anaerobiosis such as the **GasPak** disposable anaerobic indicator.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After 48 – 72 h incubation, *Clostridium difficile* will appear as flat to low umbonate, yellow colonies with a ground glass-like appearance and a slightly filamentous edge. *C. difficile* colonies may be surrounded by a yellow zone of about 2 – 3 mm, depending on colony size and incubation time. Growth may be examined with a long-wave UV light for yellow fluorescence within 1 h of removal from the anaerobic atmosphere. After exposure to air, colonies may become nonviable, which is usually accompanied by reversal of the color change to pink and a loss of fluorescence. Since some facultative anaerobic organisms potentially could produce reactions similar to *C. difficile*, it is recommended that an aerobically incubated plate be utilized in order to confirm that the isolate is an obligate anaerobe.

XI LIMITATIONS OF THE PROCEDURE

This prepared plated medium is intended for primary isolation. 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. Some species of clostridia (e.g., *butyricum*, *histolyticum*, *innocuum*, *sordellii*, and *subterminale*) may grow on this medium and produce yellow colonies and fluorescence. Also, the isolation of *Clostridium difficile* should not be relied upon for etiologic diagnosis of pseudomembranous colitis.¹⁶ Other tests, such as **ColorPAC™ C. difficile** Rapid Toxin A Test, the rapid latex agglutination test for *C. difficile* antigens (**Culturette™ CDT™ Kit**), or other toxin assays, along with clinical observations should be used.¹⁷ Appropriate references may be consulted for further information.¹⁸⁻²³

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.

XII PERFORMANCE CHARACTERISTICS

A clinical study evaluated 800 stool specimens comparing recovery on CDSA with CCFA. Of the 100 *C. difficile* positive specimens, CDSA recovered 98 (98%) compared to 86 (86%) recovery on CCFA. The selectivity of CDSA was superior to CCFA. CDSA had 15% less normal flora than CCFA. Positive cultures were compared for colony size, morphology, and amount of

growth. *C. difficile* colonies were larger on CDSA (7.30 mm) than CCFA (5.78 mm). Colony morphology was similar on both media. CDSA had a 10% increase in the amount of *C. difficile* recovered compared to CCFA.


Selectivity was evaluated in-house using 43 non-clostridia (clinical and ATCC) strains representing organisms found in stool flora and 53 strains of various *Clostridium* species (clinical and ATCC) including 9 strains of *C. difficile*. Of the 43 non-clostridia strains, 32 were completely inhibited. Partial inhibition was seen with *Candida* species, *Enterobacter aerogenes*, *Enterococcus* spp., *Lactobacillus* spp., *Pseudomonas* spp., and *Serratia* spp. Colonies of each of these species were not yellow nor had typical morphology and were easily distinguished from *C. difficile*. Thirty-five of the 44 *Clostridium* non-difficile species were completely inhibited. Growth suggestive of *C. difficile* occurred with *C. butyricum*, *C. histolyticum*, *C. innocuum*, *C. sordellii*, and *C. subterminale*. Recovery was equivalent on CDSA and CCFA. Clinical strains had increased break-through compared to ATCC strains of the same species. Repeat testing using ATCC strains and clinical strains of each species confirmed inhibition of ATCC strains, except *C. butyricum* and *C. sordellii*, and recovery of the indicated species with the clinical strains. All *C. difficile* strains were recovered and had typical morphology.


XIII AVAILABILITY

Cat. No.	Description
222228	BBL™ <i>Clostridium difficile</i> Selective Agar, Pkg. of 10 plates.

XIV REFERENCES

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