

## CLOSTRIDIUM DIFFICILE MEDIA

**PRODUCT:**

**Plate Media:**

Cefoxitin-Cycloserine Fructose Agar, CCFA, item no. P1475  
Difficile Agar, item no. P1520

**PURPOSE:**

Clostridium difficile media are used for the selective isolation of *Clostridium difficile* from clinical specimens; Cefoxitin-Cycloserine Fructose Agar (CCFA) also serves as a differential medium.

**PRINCIPLE:**

*Clostridium difficile* was isolated in 1935 by Hall and O'Toole<sup>6</sup> and has been isolated from soil, water, animal intestinal flora, the vagina and urethra of humans, the feces of many healthy infants, and in small numbers in the feces of adults. It is also resident in the hospital environment, which can lead to colonization of hospital patients. *C. difficile* was believed nonpathogenic for humans until the late 1970's when, due to the work of Bartlett et al. and Fekety et al., it was implicated as the causative agent of an antibiotic-associated diarrhea and pseudomembranous colitis. When normal gut flora is suppressed by antimicrobial or antimetabolite therapy, *C. difficile* is able to multiply and produce toxins which causes diarrhea and/or produces plaques on the cellular lining of the colon, which is indicative of the inflammatory process of pseudomembranous colitis.<sup>3</sup>

Clostridium difficile media assist in the recovery of *C. difficile*; they consist primarily of a brain heart infusion base which supports the growth of *C. difficile*, and cycloserine and cefoxitin, which inhibit the growth of other microbial flora.<sup>3</sup> CCFA also contains fructose and egg yolk; *C. difficile* metabolizes fructose, forming acid by-products, which cause the neutral red indicator in the media to change from orange to yellow. Colonies of *C. difficile* form a golden yellow fluorescence on CCFA, which is sufficient for presumptive identification.<sup>4</sup>

**FORMULAS:**

Approximate, per liter of deionized filtered water.

- (1) **Cefoxitin-Cycloserine Fructose Agar (CCFA):**
- |                                      |           |
|--------------------------------------|-----------|
| Brain Heart Infusion .....           | 10.00 g   |
| Peptic Digest of Animal Tissue ..... | 8.75      |
| Pancreatic Digest of Casein .....    | 8.75      |
| Dextrose .....                       | 2.00      |
| Sodium Chloride .....                | 5.00      |
| Disodium Phosphate .....             | 2.50      |
| Fructose .....                       | 6.00      |
| Agar .....                           | 15.00     |
| Cycloserine .....                    | 250.00 mg |
| Cefoxitin .....                      | 10.00     |
| Neutral Red .....                    | 30.00     |
| Egg Yolk Suspension .....            | 70.00 ml  |
| Final pH 7.4 ± 0.2 at 25°C           |           |

- (2) **Difficile Agar:**  
Same as (1) above except the elimination of the Fructose, Neutral Red, and Egg Yolk Suspension, and also contains 50.0 ml of Sheep Blood.

**PRECAUTIONS:\***

For in vitro diagnostic use. Observe approved biohazard precautions.

**Storage:** Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

**Limitations:** The isolation of *C. difficile* alone does not necessarily implicate it as the causative agent of gastrointestinal disease. Some authors have recommended culture, toxin assay, and sigmoidoscopy or colonoscopy for definitive diagnosis.<sup>8</sup> *Staphylococcus aureus* and enterotoxigenic *Clostridium perfringens* have been implicated as the causative agents of antibiotic-associated diarrhea and pseudomembranous colitis, although infrequently.

Up to 30% of hospitalized adults may be colonized with *C. difficile* and have no evidence of gastrointestinal disease.<sup>7</sup>

Other microbes may grow on clostridium difficile media, but most that do grow are much smaller.

As with all selective media, growth of some strains of *C. difficile* may be inhibited.

Cycloserine and cefoxitin may alter the cellular morphology of *C. difficile*, causing the cells to elongate and a decrease in the spores. Single passage onto blood agar may restore typical morphology.

#### PROCEDURE:\*

**Specimen Collection:** Information on specimen collection is found in standard reference material. In general, specimens should be protected from extremes of heat and cold and should be delivered to the laboratory immediately. *C. difficile* is one of the most oxygen-sensitive of the clinically significant anaerobes. For best recovery, transport specimens anaerobically and inoculate clinical material in an anaerobe chamber.<sup>2</sup>

**Method of Use:** Prior to inoculation, the medium should be brought to room temperature. Place 2-3 drops of liquid stool, biopsy material, or lumen contents onto the plate medium and streak the inoculum so as to obtain isolated colonies. Incubate anaerobically for 48 hours and examine for morphologically typical colonies.

**Interpretation: Colonies of *Clostridium difficile* appear as the following:**

<b>Difficile Agar:</b>	<b>Cefoxitin-Cycloserine Fructose Agar (CCFA):</b>
2-4-mm, grey, translucent colonies	4-mm, yellow, rhizoid-edged colonies
Slightly raised, rhizoid margins	Crystalline, internal structure
Crystalline, internal speckling	Yellow-green fluorescence under UV light.
	Odor similar to horse manure

Definitive identification is made after biochemical and/or metabolic product determination. See appropriate references.

**Material Required but Not Provided:** Standard microbiological supplies and equipment such as loops, needles, incubators, incinerators, transport media, and staining reagents are not provided.

#### QUALITY CONTROL:\*

##### Microorganisms Used (ATCC#):

*Clostridium difficile* (9689); (17858)  
*Clostridium perfringens* (13124)  
*Bacteroides fragilis* (25285)  
*Escherichia coli* (25922)

##### Expected Results:

Growth, yellow rhizoid-edged colonies on CCFA  
 Inhibition  
 Inhibition  
 Inhibition

**User Quality Control:** Check for signs of contamination and deterioration.

#### BIBLIOGRAPHY:

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\* For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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