CLOSTRIDIUM DIFFICILE AGAR

INTENDED USE
Remel Clostridium Difficile Agar is a solid medium recommended for use in qualitative procedures for selective and differential isolation of *Clostridium difficile*.

SUMMARY AND EXPLANATION
Clostridium difficile causes gastrointestinal infections in humans that range in severity from asymptomatic colonization to severe diarrhea, antibiotic-associated diarrhea, and pseudomembranous colitis (PMC).[1] Nosocomial infection, both symptomatic and asymptomatic, occurs through transient cross-infection of *C. difficile* on the hands of healthcare workers as well as through contact with contaminated environmental surfaces. In 1979, George et al. isolated *C. difficile* using CCFA Medium, a modification of McClung Toabe agar.[2,3] Levett described Clostridium Difficile Agar which is a modification of CCFA Medium with an egg yolk agar base and reduced concentrations of cycloserine and cefoxitin.

PRINCIPLE
Proteose peptone supplies amino acids and other nitrogenous compounds necessary for the growth of anaerobic bacteria, including *C. difficile*. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. Fructose is an energy source. Monopotassium and disodium phosphates are buffering agents which maintain the pH of the medium. Clostridium Difficile Agar is both selective and differential. The growth of *C. difficile* raises the pH of the medium causing the neutral red indicator to turn a yellow color; this can be observed in the colonies and the surrounding medium. *C. difficile* also produces a characteristic yellow fluorescence which can be observed under longwave ultraviolet light. Egg yolk reduces the toxic effect of organic peroxides which may accumulate in the medium and serves as a substrate for detection of lecithinase and lipase activity. Some species of *Clostridium* produce lecithinase and/or lipase; *C. difficile* does not. Cycloserine and cefoxitin are selective agents. Cycloserine is active against *Escherichia coli,* other gram-negative bacilli, and streptococci. Cefoxitin is a broad-spectrum antibiotic which is active against a variety of gram-positive and gram-negative bacteria, with the exception of *Enterococcus faecalis* and *C. difficile*. Agar is a solidifying agent.

REAGENTS (CLASSICAL FORMULA)*

Proteose Peptone	40.0 g	Magnesium Sulfate	0.1 g
Fructose	6.0 g	Neutral Red	0.03 g
Disodium Phosphate	5.0 g	Cefoxitin	0.016 g
Sodium Chloride	2.0 g	Egg Yolk Suspension	100.0 ml
Monopotassium Phosphate	1.0 g	Agar	20.0 g
Cycloserine	0.25 g	Demineralized Water	900.0 ml

pH 7.6 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Prior to use, reduce the plates for a minimum of 24 hours by placing them in an anaerobic jar at room temperature.
2. Inoculate specimens for anaerobic culture on both selective and nonselective media.
3. Incubate anaerobically at 33-37°C for 48-72 hours.
4. Following incubation, examine the plate for flat, circular colonies with filamentous edges that demonstrate a yellow zone extending 2-3 mm from the edge of the colony.
5. Inspect suspicious colonies under longwave ultraviolet light for yellow fluorescence.
6. Confirm anaerobic growth by subculture of colonies representative of *C. difficile* to a blood agar plate incubated at 33-37°C in ambient air.
7. Consult appropriate references for additional tests to confirm the presence of *C. difficile*.[5,6]

QUALITY CONTROL
All lot numbers of Clostridium Difficile Agar have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Clostridium difficile ATCC® 9689	Anaerobic, 48-72 h @ 33-37°C	Growth, yellow colonies, yellow zone, yellow fluorescence
Bacteroides fragilis ATCC® 25285	Anaerobic, 48 h @ 33-37°C	Inhibition (partial to complete)
Clostridium perfringens ATCC® 13124	Anaerobic, 48 h @ 33-37°C	Inhibition (partial to complete)
Escherichia coli ATCC® 25922	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)

LIMITATIONS
1. Other organisms may grow on this medium, but usually do not have the characteristics described of *C. difficile*.[7]
2. Colonies of *C. difficile* isolated on this medium from specimens containing mixed microbial flora (e.g., feces) are slightly smaller than when grown in pure culture.[3,7]
3. The yellow fluorescence of *C. difficile* is detectable after 24 hours and persists for 5-6 days.[7]
4. The Gram stain morphology of *C. difficile* isolated on this medium may be atypical. Cycloserine and cefoxitin alter cellular morphology causing marked elongation of cells and loss of spores.[3,7]

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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