Product Insert

CYCLOSERINE-CEFOXITIN FRUCTOSE AGAR WITH HORSE BLOOD AND TAUROCHOLATE (CCFA-HT)

Products

AS-2136 Cycloserine-Cefoxitin Fructose Agar with Horse Blood and Taurocholate (CCFA-HT) 1 plate / pkg

Intended Use

Cycloserine-Cefoxitin Fructose Agar with Horse Blood and Taurocholate (CCFA-HT) is an enriched selective media used for the isolation and presumptive identification of *Clostridium difficile*, a recognized cause of pseudomembraneous (antimicrobial agent-associated) colitis.

Summary

CCFA-HT is an enriched selective media used for the isolation of *Clostridium difficile*. The basic nutritive base consists of animal peptones and fructose and is supplemented with cefoxitin and cycloserine at concentrations that inhibit the growth of most normal fecal flora. Cycloserine will inhibit gram-negative bacteria, while cefoxitin will inhibit both gram-positive and gram-negative organisms. *Clostridium difficile* is not inhibited on the CCFA-HT media and when grown, will exhibit a characteristic colonial morphology, fluorescence, and smell. Horse blood is added to stimulate growth. Sodium Taurocholate is added to help in the germination of spores. This media is prepared, dispensed, and packaged under oxygen-free conditions to prevent the formation of oxidized products prior to use.

Formulation*

Proteose Peptone	40.00	g
Sodium Phosphate Dibasic	5.00	g
Potassium Phosphate Monobasic	1.00	g
Sodium Chloride	2.00	g
Magnesium Sulfate Heptahydrate	0.20	g
Fructose	6.00	g
Agar	15.00	g
Sodium Taurocholate	1.00	g
Defibrinated Horse Blood	70.00	mL
Cycloserine (10.0% solution)	2.50	mL
Cefoxitin (1.56% solution)	1.00	mL
DI Water	1.00	L

Final pH: 7.3 ± 0.3 at 25^{0}C
Final weight: 17.0 g ± 1.7 g

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

Precautions

For *IN VITRO DIAGNOSTIC USE* only. Utilize approved biohazard precautions and aseptic technique when using this product. This product is for use only by properly trained and qualified personnel. Sterilize all biohazard waste prior to disposal.

Storage and Shelf Life

Storage: Upon receipt, store at 2 – 8^{o}C in original packaging until used. Avoid overheating or freezing. Do not use media if there are signs of deterioration (shrinking, cracking, or discoloration due to oxidation of media) or contamination. The expiration date

applies to the product in its original packaging and stored as directed. Do not use product past the expiration date shown on the container.

Shelf Life: 90 days from date of manufacture

Procedure

Specimen Collection: Protect specimens for anaerobic culture from oxygen during collection, transportation, and processing. Consult appropriate references for detailed instructions concerning collection and transportation of anaerobes.

Methods for Use: CCFA-HT should be inoculated directly with clinical specimen or from a broth that has been previously inoculated from a clinical specimen. Streak plates with inoculum to obtain isolated colonies and immediately place in an anaerobic atmosphere, incubating at 35-37°C for 18-48 hours. Quantitation of *C. difficile* in a specimen may be clinically useful, which can be achieved by thoroughly mixing a serial 10-fold dilution of the specimen in an anaerobic environment followed by plating the dilutions onto CCFA-HT media. Detailed instructions for processing anaerobic cultures can be found in the appropriate references.

Materials Required, But Not Provided

Standard microbiological supplies and equipment such as loops, saline blanks, slides, staining supplies, microscope, incinerator / autoclave, incubators, anaerobic chamber / anaerobic jars, other culture media, and serological / biochemical reagents.

Interpretation of Results

If used properly, CCFA-HT supports good growth of species of *C. difficile*. After 24-48 hours, most colonies of *C. difficile* are large, grey, slightly filamentous, and low umbonate to flat. These colonies fluoresce golden yellow/chartreuse under long-wavelength UV light.

Limitations

CCFA-HT will not provide complete information for identification of bacterial isolates. Rare strains of *C. difficile* may be inhibited. Plates must be examined no later than after 48 hours of incubation for optimal selectivity, because after 3 – 5 days of incubation, significant numbers of colonies other than *C. difficile* may grow. At 48 hours, colonies of most other organisms (e.g. *Lactobacilli, Clostridia,* and yeast), which may grow, are very small (pinpoint to 0.5 mm in diameter) and do not fluoresce golden-yellow. A test for aerotolerance should be used to confirm that each colony type is an obligate anaerobe. Consult reference materials for additional information.

Quality Control

The following organisms are routinely used for quality control performance testing at Anaerobe Systems.

Organism Tested	ATCC #	Results	Time	Special Reaction
Bacteroides fragilis	25285	No Growth		
Enterococcus faecalis	29212	No Growth		
Clostridium sporogenes	3584	No Growth		
Clostridium beijerinckii	8260	No Growth		
Proteus mirabilis	12453	No Growth		
Clostridium perfringens	13124	No Growth		
Clostridium sordellii	9714	No Growth		
Clostridium innocuum	14501	Inhibited to No Growth		
Clostridium difficile	9689	Growth	24 hrs	Chartreuse fluorescence
Clostridium difficile	700057	Growth	24 hrs	Chartreuse fluorescence

User Quality Control: The final determination to the extent and quantity of user laboratory quality control must be determined by the end user.

If sterility testing is to be performed on this product, a representative sample of the lot(s) should be incubated anaerobically and aerobically for 48 – 96 hours.

If the nutritive/inhibitory capacity of this media is to be tested for performance, it is recommended that the following ATCC organisms be evaluated for growth/inhibition.

Organism	ATCC #	Expected Growth
B. fragilis	25285	Inhibited
E. coli	25922	Inhibited
S. aureus	25923	Inhibited
C. difficile	9689	24 hours

Physical Appearance: CCFA-HT should be opaque burgundy-red in color.

References

1. Dowell, V. R., Jr., G. L. Lombard, F. S. Thompson and A. Y. Armfield. 1977. *Media for the Isolation, Characterization, and Identification of Obligately Anaerobic Bacteria*. USDHHS, CDC, Atlanta, GA 30333.

2. Holdeman, L. V., F. P. Cato and W. E. C. Moore. 1987. *Anaerobe Laboratory Manual*. Virginia Polytechnic Institute and State University. Blacksburg, VA 24061.

3. Jousimies-Somer, H. R., P. Summanen, D. M. Citron, E. J. Baron, H. M. Wexler and S. M. Finegold. 2002. *Wadsworth – KTL Anaerobic Bacteriology Manual*. Star Publishing Co., Belmont, CA 94002.

4. CLSI. *Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard- Third Edition.* (2004). CLSI document M22-A3. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898.

5. George, W. L., V.L. Sutter, D. Citron, S. Finegold. 1979. Selective and Differential Medium for Isolation of *Clostridium difficile*. *Journal of Clinical Microbiology*. 9:214-219.

6. Wilson KH, Kennedy MJ, Fekety FR: Use of Sodium Taurocholate to Enhance Spore Recovery on a Medium Selective for *Clostridium difficile*. Journal of Clinical Microbiology 1982, 15(3): 443-446.

7. Marler LM, Siders JA, Wolters LC, Pettigrew Y, Skitt BL, Allen SD: Comparison of Five Cultural Procedures for Isolation of *Clostridium difficile* from Stools. Journal of Clinical Microbiology 1992, 30(2): 514-516.

8. Edelstein, Martha. "Isolation and Identification of *Clostridium difficile*; Tissue Culture and Cytotoxicity Assay." Clostridium difficile: Its Role in Intestinal Disease. Eds. Rolfe RD, Finegold SM. San Diego: Academic Press Inc, 1988. 287-307.

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