

Product Insert

CYCLOSERINE CEFOXITIN FRUCTOSE AGAR WITH LYSOZYME (CCFA-L)

Products

AS-2133 Cycloserine Cefoxitin Fructose Agar with Lysozyme (CCFA-L)

4 plates / pkg

Intended Use

Cycloserine Cefoxitin Fructose Agar with Lysozyme (CCFA-L) is an enriched, selective, and differential media used for the isolation and presumptive identification of *Clostridium difficile*, a recognized cause of pseudomembranous (antimicrobial agent-associated) colitis.

Summary

CCFA-L is an enriched, selective, and differential media 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 CCFA-L media, and when grown on this media, will exhibit a characteristic yellow, ground-glass colonial morphology. Neutral red is added as a pH indicator and lysozyme is added to aid in the germination of spores. The presence of *C. difficile* will turn the indicator from pink/orange to yellow, due to the utilization of amino acids by the organism causing an increase in the pH. 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 |
| Neutral Red (0.3% solution) | 3.00 | mL |
| Cycloserine (10.0% solution) | 2.50 | mL |
| Cefoxitin (1.56% solution) | 1.00 | mL |
| Lysozyme (1.0% solution) | 1.00 | mL |
| DI Water | 1.00 | L |

Final pH: 7.2 ± 0.2 at 25°C

Final weight: 16.0 g ± 1.6 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°C in original container 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 transport of anaerobes.

Methods for Use: Remove CCFA-L from 2 - 8°C environment and allow to come up to room temperature. CCFA-L should be inoculated directly with clinical specimens or from a broth that has been previously inoculated from a clinical specimen. Streak plates with inoculum to obtain isolated colonies and immediately place into 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-L 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, other culture media, and serological / biochemical reagents.

Interpretation of Results

If used properly, CCFA-L supports good growth of members of *C. difficile*. After 24-48 hours, most colonies of *C. difficile* are large, circular, and yellow, with the yellow coloration extending 2 –3 mm beyond the colony into the initially pink/orange media. These colonies fluoresce golden yellow/chartreuse under long-wavelength UV light. Most other bacteria are inhibited on this media. At 48 hours, colonies of most other organisms (e.g. *Lactobacilli* and yeast), which may grow, are very small (pinpoint to 0.5 mm in diameter) and do not fluoresce golden-yellow.

Limitations

CCFA-L 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. Significant numbers of colonies, other than *C. difficile*, may grow after 48 hours of incubation. 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 innocuum | 14501 | No Growth | | |
| Clostridium sordellii | 9714 | No Growth | | |
| Clostridium difficile | 9689 | Growth | 24 hrs | Yellow coloration, chartreuse fluorescence |
| Clostridium difficile | 700057 | Growth | 24 hrs | Yellow coloration, 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 | Special Reaction |
|--------------|--------|-----------------|--------------------------------------------|
| B. fragilis | 25285 | Inhibited | |
| E. coli | 25922 | Inhibited | |
| S. aureus | 25923 | Inhibited | |
| C. difficile | 9689 | 24 hours | Yellow coloration, chartreuse fluorescence |

Physical Appearance: CCFA-L should appear translucent pink/orange in color.

References

1. Dowell, V. R., Jr. and T. M. Hawkins. 1987. *Laboratory Methods in Anaerobic Bacteriology*. CDC Laboratory Manual. USDHHS CDC. Atlanta, GA 30333.
2. Dowell, V. R., Jr. and G. L. Lombard. 1981. *Presumptive Identification of Anaerobic Non-sporeforming Gram-negative Bacilli*. USDHHS, CDC. Atlanta, GA 30333.
3. 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.
4. Holdeman, L. V., F. P. Cato and W. E. C. Moore. 1987. *Anaerobe Laboratory Manual*. Virginia Polytechnic Institute and State University. Blacksburg, VA 24061.
5. 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.
6. 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.
7. Wilcox MH, Fawley WN, Parnell P: Value of lysozyme agar incorporation and alkaline thioglycollate exposure for the environmental recovery of *Clostridium difficile*. *Journal of Hospital Infection* 2000, 45: 65-69.
8. 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.

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