
anaeroGRO™
Pre-Reduced Anaerobic Culture Media

# CYCLOSERINE-CEFOXITIN FRUCTOSE AGAR (CCFA)

| | | |
|---|---|---|
| Cat. no. AG501 | Cycloserine-Cefoxitin Fructose Agar*, 15x100mm Plate, 18ml | 1 plate/pouch |

* All AnaeroGRO™ plated media is provided in standard 15x100mm monoplates or biplates. Each plate or set of plates is packaged in an oxygen-free gas flushed foil pouch containing a desiccant and an oxygen scavenger sachet.

## INTENDED USE

Hardy Diagnostics AnaeroGRO™ Cycloserine-Cefoxitin Fructose Agar (CCFA) is an enriched selective and differential medium recommended for the isolation and cultivation of *Clostridium difficile* from fecal specimens. *C. difficile* is a recognized cause of intestinal infections and pseudomembranous colitis following antibiotic therapy.[5,7]

## SUMMARY

*Clostridium difficile* is considered the most common cause of pseudomembranous colitis (PMC) or antibiotic-associated diarrhea (AAD) frequently infecting patients on recent antibiotic therapy. Other risk factors associated with PMC include advancing age and recent major surgery, and this organism is easily transmitted from patient-to-patient in hospitals, health care settings, and long-term care facilities.

Early studies on PMC revealed that traditional media selective for the growth of clostridia were inhibitory to *C. difficile*; however, George et al. developed a selective and differential growth medium containing cycloserine, cefoxitin, fructose, and egg yolk (CCFA) to facilitate the isolation and differentiation of *C. difficile* from fecal specimens.[2,4,10]

Ingredients in CCFA, such as animal peptones and fructose, have been optimized to improve recovery of *C. difficile* and the medium contains neutral red as a pH indicator. As amino acids are utilized by the organism, the pH increases resulting in a color change in the medium from orange to yellow. CCFA is also made selective by the addition of cycloserine and cefoxitin. Cycloserine inhibits *Escherichia coli* while partially inhibiting other gram-negative bacilli and streptococci. Cefoxitin is a broad spectrum antimicrobial that inhibits gram-positive and gram-negative microorganisms, excluding *Enterococcus faecalis* and *Clostridium difficile*.

On CCFA medium, *C. difficile* colonies appear as yellow, with a ground-glass appearance, and are circular with a slightly filamentous edge, flat to low with a rounded elevation, lipase and lecithinase negative and will exhibit a characteristic golden-yellow fluorescence when examined under long-wave ultraviolet light.[4,5,7,9]

AnaeroGRO™ Cycloserine-Cefoxitin Fructose Agar (CCFA) is packaged in an oxygen-free, reduced state to prevent the formation of toxic oxidized by-products that may damage obligate anaerobes and inhibit the growth of more fastidious species. Culture media that is exposed to environmental oxygen leads to a build-up of reactive oxygen species (ROS) that initiate damaging free radical reactions, which inhibit the growth of anaerobic bacteria. Therefore, ingredients have been added to the AnaeroGRO™ media to neutralize the growth inhibiting effects of peroxide and other reactive oxygen species (ROS) that may develop during the medium's brief exposure to oxygen after it is sterilized and before it is packaged in an oxygen-free environment.

## FORMULA

Ingredients per liter of deionized water:*

| | |
|---|---|
| Proteose Peptone | 40.0gm |
| Fructose | 6.0gm |
| Disodium Phosphate | 5.0gm |
| Sodium Chloride | 2.0gm |
| Reducing Agents/Peroxide Inhibitors | 1.5gm |
| Monopotassium Phosphate | 1.0gm |
| Magnesium Sulfate | 0.1gm |
| Neutral Red | 0.03gm |
| Cycloserine | 500.0mg |
| Cefoxitin | 16.0mg |
| Agar | 15.0gm |

Final pH 7.4 +/- 0.2 at 25ºC.

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

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

The plates must be inoculated **immediately** after opening the AnaeroGRO™ pouch. After inoculation, the plates must be placed **immediately** into an anaerobic atmosphere (pouch, jar, or chamber) to ensure optimal growth of anaerobic bacteria.

Refer to the document "Storage" for more information.

## PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not

ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

## PROCEDURE

Specimen Collection: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat, cold and oxygen exposure. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate anaerobic transport media (Cat. no. 28050510 or S120D) and refrigerated until inoculation. Consult listed references for information on specimen collection.[1-9]

Method of Use:

1. Open the AnaeroGRO™ pouch just prior to use and apply 2-3 drops of liquid stool, biopsy material, or lumen contents directly onto the medium. Streak the inoculum to obtain isolated colonies. An inoculum can also be obtained from a broth, such as Thioglycollate Broth with H & K (Cat. no. AG22H), which has been previously inoculated with clinical material.

2. Immediately following inoculation, place the medium in an anaerobic atmosphere and incubate at 35-37ºC. for 18-48 hours. Use an indicator of anaerobiosis, such as resazurin (Cat. no. BR55).

3. Observe daily for characteristic colonial morphology.

## INTERPRETATION OF RESULTS

*Clostridium difficile* is differentiated by the growth of large colonies (approximately 4mm in diameter) that are circular with a slightly filamentous edge, low undulate to flat in profile, ground-glass in appearance, and yellow with yellow coloration extending 2-3mm beyond the colony into the medium. Colonies of *C. difficile* typically fluoresce golden-yellow under a long-wave ultraviolet light.[4,5,7,9]

Microorganisms other than *C. difficile* may grow on CCFA, but do not produce a yellow color change in the medium nor show golden-yellow fluorescence under long-wave UV light. Such organisms are generally smaller than colonies of *C. difficile*.

Refer to the *Wadsworth-KTL Anaerobic Bacteriology Manual* or other texts for more information on the identification of anaerobes.[7]

## LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

The plates must be inoculated **immediately** after opening the AnaeroGRO™ pouch. After inoculation, the plates must be placed **immediately** into an anaerobic atmosphere (pouch, jar, or chamber) to ensure optimal growth of anaerobic bacteria.

Rare strains of *C. difficile* may be inhibited on CCFA.

For optimal results, plates should not be examined beyond 48 hours of incubation. Extended incubation may result in significant growth of colonies other than *C. difficile*.

Organisms other than *C. difficile* may grow on CCFA, but can be distinguished by their morphological differences.

An aerotolerance test should be performed on colonies suspected to be *C. difficile*. Aerotolerance testing is used to confirm that each colony type is an obligate anaerobe.

Failure to cultivate and/or isolate obligate anaerobes may be due to the following:

1. Exposure of specimen to oxygen during transport or processing.

2. Overgrowth of aerobic, facultative organisms, or normal flora. Overgrowth can occur in transport media, Thioglycollate broth, or on non-selective plated media. This can be controlled by avoiding normal flora during specimen collection and by utilizing selective plated media.

3. Leaks in the anaerobic incubation system; e.g. faulty O-rings or vents.

4. Failure to use an anaerobic indicator (such as resazurin) to monitor for complete anaerobiosis.

5. Anaerobic gas mixture contains toxic gas, oxygen; or does not include $CO_2$, which is necessary for some anaerobes.

6. Failure to use a non-selective medium as part of the primary specimen set-up, since some fastidious anaerobes are inhibited by selective media.

7. Failure to perform quality control of the media and processing procedures.

8. Failure to incubate cultures for extended periods of time. Some fastidious anaerobes are slow growers (such as *Porphyromonas* and *Actinomyces* spp.), especially if present in small numbers, and may require 5 to 7 days of incubation in order to be visible on plated media.

9. Exposure of developing colonies on plated media to air, especially when opening jars to check for growth. Anaerobes are most sensitive to oxygen during the log phase of growth. Do not open jars or pouches at less than 48 hours of incubation (except when incubating BBE or EYA plates, since organisms that are selected for on these plates grow rapidly). Some fastidious anaerobes may lose viability with only 15 minutes of exposure to oxygen.
Plates incubated in anaerobic chambers or unopened pouches can be inspected at 24 hours, since the plates are not exposed to oxygen during inspection.

Refer to the document "Limitations of Procedures and Warranty" for more information.

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, transport media (Cat. no. 28050510 or S120D), incubators, incinerators, anaerobic culture materials, such as gas

generators (Cat. no. AN25US), compact systems (Cat. no. AN010C), sealing clips (Cat. no. AN005C), chambers, jars (Cat. no. 16000), and oxygen indicators (Cat. no. BR55), etc., as well as serological and biochemical reagents, are not provided.

## QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

| Test Organisms | Inoculation Method* | Incubation | | | Results |
|---|---|---|---|---|---|
| | | Time | Temperature | Atmosphere | |
| *Clostridium difficile* ATCC® 9689** | A | 24-48hr | 35°C | Anaerobic | Growth; large, yellow colonies that fluoresce golden-yellow under UV light |
| *Clostridium perfringens* ATCC® 13124** | B | 24-48hr | 35°C | Anaerobic | Partial to complete inhibition |
| *Bacteroides levii* ATCC® 29147 | B | 24-48hr | 35°C | Anaerobic | Partial to complete inhibition |
| *Bacteroides fragilis* ATCC® 25285 | B | 24-48hr | 35°C | Anaerobic | Partial to complete inhibition |
| *Fusobacterium necrophorum* ATCC® 25286 | B | 24-48hr | 35°C | Anaerobic | Partial to complete inhibition |
| *Peptostreptococcus anaerobius* ATCC® 27337 | B | 24-48hr | 35°C | Anaerobic | Partial to complete inhibition |
| *Escherichia coli* ATCC® 25922 | B | 24-48hr | 35°C | Aerobic | Partial to complete inhibition |
| *Staphylococcus aureus* ATCC® 25923 | B | 24-48hr | 35°C | Aerobic | Partial to complete inhibition |

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

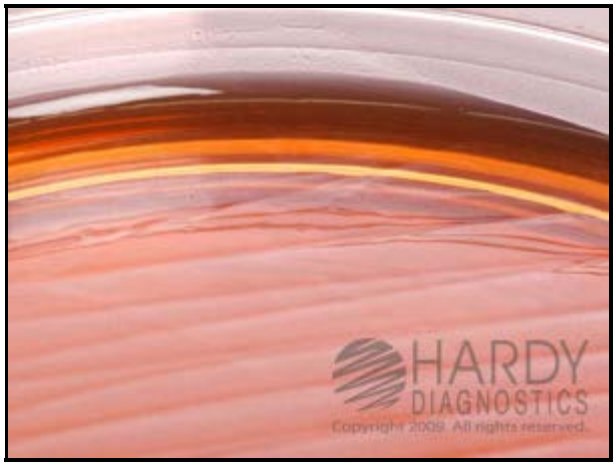
## USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

## PHYSICAL APPEARANCE

AnaeroGRO™ Cycloserine-Cefoxitin Fructose Agar (CCFA) should be clear, and pinkish-beige in color.


*Clostridium difficile* (ATCC® 9689) colonies on AnaeroGRO™ CCFA Agar (Cat. no. AG501) under UV light. Incubated anaerobically for 48 hours at 35ºC.




*Clostridium perfringens* (ATCC® 13124) growth inhibited on AnaeroGRO™ CCFA Agar (Cat. no. AG501) under ambient light. Incubated anaerobically for 48 hours at 35ºC.

## REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory,* Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Dowell, V.R., Jr. 1981. Media for the Selective Isolation of *Clostridium difficile*. Les Anaerobes Microbiologie-Pathologie. Symposium International. New York.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

4. George, W.L., V.L. Sutter, D. Citron, and S.M. Finegold. 1979. Selective and Differential Medium for Isolation of *Clostridium difficile*. *J. Clin. Microbiol.*; 9(2):214-219.

5. Holdeman, L.V., E.P. Cato, and W.E.C. Moore. 1977. *Anaerobe Laboratory Manual*, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg, VA.

6. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

7. Jousimies-Somer, H.R., S.P. Citron, D. Baron, E.J. Wexler, and H.M. Finegold. 2002. *Wadsworth-KTL Anaerobic Bacteriology Manual*, 6th ed. Star Publishing Company, New York, N.Y.

8. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology,* J.B. Lippincott Company, Philadelphia, PA.

9. Jorgensen., et al. *Manual of Clinical Microbiology,* American Society for Microbiology, Washington, D.C.

10. Wust, J., N.M. Sullivan, U. Hardegger, and T.D. Wilkins. 1982. Investigation of an Outbreak of Antibiotic-Associated Colitis by Various Typing Methods. *J. Clin. Microbiol.*; 16:1096-1101.



IFU-10027[A]


1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: www.HardyDiagnostics.com
Email: TechService@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.



HDQA 2207A [C]