IFU

Instructions for Use

CAMPY CEFEX AGAR, MODIFIED

Cat. no. A122	Campy Cefex Agar, Modified, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Campy Cefex Agar, Modified plates are recommended for the selective isolation of cephalothin-resistant *Campylobacter* species such as *C. jejuni*, *C. coli*, and *C. lari* from human, animal, or food samples.

SUMMARY

Campylobacter species have been recognized as a major cause of diarrheal disease in children and adults. Originally these organisms were only associated with a variety of veterinary diseases. *Campylobacter* spp. has been characterized as among the top bacterial agents of human foodborne gastroenteritis. The organisms may also be transmitted by contaminated food or water. Poultry is a primary reservoir of *Campylobacter* spp. and studies show that prevalence may be greater than 80% in commercial chicken carcasses. 95% of human illnessess are associated with *Campylobacter jejuni*, followed by *C. coli* at 4%. Other species are involved in only 1% of infections.

Brucella Agar is a highly nutritious base and the addition of horse blood supplements the medium with X-factor (hemin) and other growth factor requirements. The addition of antimicrobials to the medium is required to suppress the growth of normal fecal flora. Cefoperazone is added to inhibit many gram-positive and gram-negative organisms, both aerobic and anaerobic. Amphotericin B is added to inhibit the growth of fungi.

Cephalothin-sensitive *Campylobacter* spp. such as *C. fetus* and *C. upsaliensis* may not be recovered on Campy Cefex Agar, Modified because it contains cefoperazone.[(2)]

FORMULA

Ingredients per 950ml deionized water:*

Brucella Agar	43.0gm
Ferrous Sulfate	0.5gm
Sodium Pyruvate	0.5gm
Sodium Bisulfite	0.2gm
Amphotericin B	0.02gm
Cefoperazone	33.0mg
Lysed Horse Blood	50.0ml

Final pH 7.2 +/- 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), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat and freezing.

The expiration date 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 incubation times as stated below.

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 Precautions for blood. 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." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

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 M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

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

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[1-3,5,9] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

Method of Use:

Direct Inoculation:
1. Swab: Inoculate a Campy Cefex Agar, Modified plate using the four quadrant streak technique for maximum isolation.

2. Diarrheal Stool: Inoculate a Campy Cefex Agar, Modified plate with three drops of stool and streak for isolation. At the same time, make a direct smear and look for small curved gram-negative bacilli and fecal leukocytes.

3. Solid Stool: Prepare a 1:10 suspension of stool by placing pea-sized amount into 5ml of physiological saline (0.85%). Vortex the sample. Inoculate a Campy Cefex Agar, Modified plate with three drops of this suspension, and streak for isolation.

Indirect Inoculation:
1. Swab: Place the swab into an appropriate transport medium and refrigerate overnight.

2. Diarrheal Stool: Place five drops of the specimen approximately one centimeter below the surface of an appropriate broth medium (Cat. no. K128). Refrigerate overnight.

3. Solid Stool: Prepare a 1:10 suspension of stool by placing pea-sized amount into 5ml of physiological saline (0.85%). Vortex the sample. Place five drops of the specimen approximately one centimeter below the surface of an appropriate broth medium (Cat. no. K128). Refrigerate overnight.

4. Subculturing appropriate broth medium (Cat. no. K128): Place a pasteur pipet one inch below the surface of the broth medium and withdraw a large aliquot towards the surface. Place three drops onto a Campy Cefex Agar, Modified plate and streak for isolation.

Incubate Campy Cefex Agar, Modified plates at 42ºC. for 48-72 hours in a microaerophilic atmosphere of 85% nitrogen, 10% carbon dioxide, and 5% oxygen. In addition, media may be set up in duplicate, with the second set incubated at 35-37ºC. to allow for the growth of certain *Campylobacter*species.

INTERPRETATION OF RESULTS

Campylobacter jejuni colony morphology may appear as small, mucoid, grayish, flat colonies with irregular edges and no hemolytic patterns at 24-48 hours. Colonies may also appear pink or yellowish-gray with some colonies exhibiting a tailing effect along the streak line.[3] They may also appear as round, convex, entire, glistening colonies 1-2mm in diameter.

Consult the listed references for more information regarding the identification and further testing of *Campylobacter* species.[1-3,5]

LIMITATIONS

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

Cephalothin sensitive *Campylobacter* spp. such as *C. fetus* and *C. upsaliensis* may not be recovered on Campy Cefex Agar, Modified because it contains cefoperazone.[2]

Due to the presence of dextrose in the medium, some weak oxidase reactions may occur. Testing should be performed on growth taken from a medium without dextrose, if this phenomenon occurs.

The agents in selective media may inhibit some strains of desired species or permit the growth of species they were designed to inhibit. Therefore, specimens cultured on selective media should also be cultured on non-selective media to obtain additional information and to help insure recovery of potential pathogens.

The reactions observed with Campy Cefex Agar, Modified are not sufficient to speciate organisms.

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, other culture media, slides, staining reagents, pasteur pipettes, microaerophilic atmosphere packets, incubation jars, catalysts, incinerators, and incubators, 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 Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Campylobacter jejuni ATCC® 33291	A	48hr	35°C	Microaerophilic**	Growth
Proteus mirabilis ATCC® 12453	B	48hr	35°C	Aerobic	Partial to complete inhibition
Escherichia coli ATCC® 25922	B	48hr	35°C	Aerobic	Partial to complete inhibition
Candida albicans ATCC® 10231	B	48hr	35°C	Aerobic	Partial to complete inhibition

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

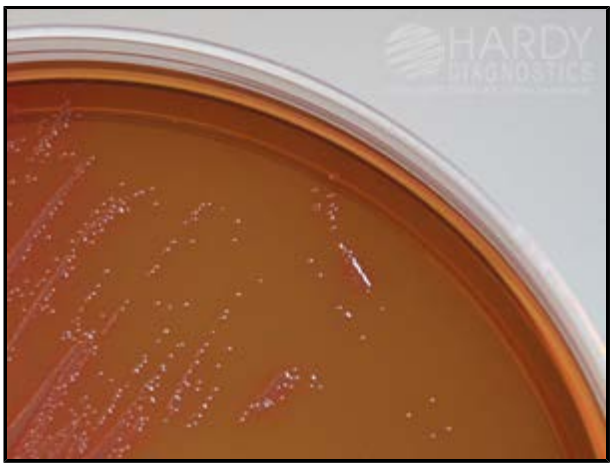
** Atmosphere of incubation is enriched with 5% O_2, 10% CO_2, and 85% N_2.

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 certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Campy Cefex Agar, Modified should appear clear, and dark red in color.



Campylobacter jejuni (ATCC® 33291) growing on Campy Cefex Agar, Modified (Cat. no. A122). Incubated microaerophilically for 48 hours at 35ºC.

Proteus mirabilis (ATCC® 12453) growth inhibited on Campy Cefex Agar, Modified (Cat. no. A122). Incubated aerobically 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. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

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

4. Blaser, M.J., et al. 1979. *Campylobacter enteritis*: clinical and epidemiologic features. *Annals of Intern. Med.*; 91:179-185.

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

6. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

8. Reller, L.B., et al. 1983. Controlled evaluation of an improved selective medium for the isolation of *Campylobacter jejuni* from human feces, Abstract, ASM Annual Meeting.

9. Oyarzabal, O.A, et al., "Evaluation of Agar Plates for Direct Enumeration of *Campylobacter* spp. from Poultry Carcass Rinses". *Applied and Environmental Microbiology*: June 2005, p. 3351-3354.

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HDQA 2207F [D]