IFU

Instructions for Use

CAMPY CVA AGAR

Cat. no. A40	Campy CVA Agar, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Campy CVA Agar Plates are recommended for the selective isolation of *Campylobacter* species from fecal specimens.

SUMMARY

Campylobacter species are microaerophilic organisms that inhabit the gastrointestinal tracts of various animals, including poultry, dogs, cats, sheep, and cattle. *C. jejuni* and *C. coli* are the most common *Campylobacter* species associated with gastrointestinal infection and are clinically indistinguishable. In fact, it is thought that approximately 5 to 10% of cases reported as being due to *C. jejuni* in the U.S. are probably due to *C. coli* . *Campylobacter lari* has also been recognized as a cause of gastroenteritis, but less frequently than *C. jejuni* . *C. jejuni* continues to be the most common enteric pathogen isolated form patients with diarrhea. Symptoms of *C. jejuni* or *C. coli* infection usually include fever, abdominal cramping, and diarrhea that lasts for several days to more than 1 week. Symptomatic infections, such as gastroenteritis, are usually self-limiting and do not require antibiotic therapy, although relapses may occur in 5 to 10% of untreated patients. Deaths attributed to *C. jejuni* infection are uncommon.[1,2,5,6]

Campylobacter infections are usually sporadic and tend to occur in the summer and early fall. Outbreaks are associated with ingestion of contaminated milk and water. Ingestion of improperly handled or under cooked food, primarily poultry products, raw milk, or contaminated water are common sources for human infections. It takes relatively few Campylobacter cells to cause illness and or symptoms of gastroenteritis in humans. It is thought that the infective dose of *C. jejuni* ranges from 500 - 10,000 cells, depending on the strain, damage to cells from environmental stresses, and the susceptibility of the host.[5,11] Infants and young children are the most susceptible. Travelers to developing countries are also at risk for Campylobacter infections.[1,2,4-6]

A number of selective media are recommended for the isolation of *C. jejuni* and *C. coli* ., including blood-free media, such as Charcoal-Cefoperazone-Deoxycholate Agar (CCDA), Charcoal-based Selective Medium (CSM). There are also blood containing media, such as Skirrow medium and Campy CVA medium. To achieve the highest yield of *Campylobacter* organisms from stool samples, a combination of media appears to be optimal, and may increase the recovery by as much as 10 to 15% over the use of a single medium. If only a single medium is used, Campy CVA is recommended. The use of cefoperazone-containing media, as opposed to cephalothin-containing media, is recommended for the primary isolation of *Campylobacter* from fecal samples (see "Limitations" section below).[1,6,8] 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.[12]

Hardy Diagnostics Campy CVA Agar contains peptamin which provides carbon, sulfur, and nitrogenous compounds required for growth. Yeast extract supplies B vitamins to the medium, and dextrose is incorporated as an energy source. Sheep blood supplements the medium with X-factor and other growth factor requirements. The addition of antimicrobials to the media 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. Vancomycin inhibits gram-positive microorganisms. Amphotericin B is incorporated to inhibit the growth of yeast.

FORMULA

Ingredients per liter of deionized water:*

Peptamin	20.0gm
Sodium Chloride	5.0gm
Yeast Extract	2.0gm
Dextrose	1.0gm
Sodium Bisulfite	0.1gm
Cefoperazone	20.0mg
Vancomycin	10.0mg
Amphotericin B	2.0mg
Sheep Blood, Defibrinated	50.0ml
Agar	17.5gm

Final pH 7.0 +/- 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.[6-8] 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.

Fecal specimens are the preferred sample for isolating *Campylobacter* species from patients with gastrointestinal infections; however rectal swabs are acceptable for cultures. A transport medium, such as Cary-Blair or Campy-Thio medium, should be used if there is a delay of more than 2 hours to the lab, and for transport of rectal swabs. Specimens received in transport medium should be processed immediately or stored at 4ºC. until processed.[1,2]

Several references suggest using the filtration method in conjunction with direct culturing on selective media. Please see listed references for procedure of the filtration method for recovering *Campylobacter* species.

Enrichment broths are used to enhance the recovery of *Campylobacter* from stool samples, such as Preston enrichment and Campy-Thio. Enrichment cultures may be beneficial when low numbers of the organisms are expected. Use of enrichment cultures as part of routine stool culture setup is probably not necessary.[1,3,7]

Method of Use:

Direct Inoculation:

1. Swab: Inoculate a Campy CVA Agar plate using the four quadrant streak technique for maximum isolation.

2. Diarrheal Stool: Inoculate a Campy CVA Agar 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 CVA Agar 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 CVA Agar plate and streak for isolation.

Incubate Campy CVA Agar 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. They may also appear as round, convex, entire, glistening colonies 1-2mm in diameter. Certain strains of *C. jejuni* may appear lightly pink or tan in color.

Consult the listed references for more information regarding the identification and further testing of *Campylobacter* species.[1-3,7]

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.

Campylobacter species are not easily visualized with the safranin counterstain normally used in the Gram stain procedure; therefore carbolfuchsin or 0.1% aqueous basic fuchsin can be used as the counterstain, or extending the staining time of the safranin to at least 10 minutes can improve the intensity of the stain.[1,6]

Most *Campylobacter* species require a microaerobic atmosphere containing approximately 5% O_2, 10% CO_2, and 85% N_2 for optimal recovery. The concentration of oxygen generated in candle jars is not optimal for the isolation of *Campylobacter* spp. and should not be used.[1]

Certain *Campylobacter* species, such as *C. sputorum* , *C. concisus* , *C. mucosalis* , etc., may require hydrogen for primary isolation and growth[1]

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 antimicrobial agents that are present in some Campylobacter Selective medias, such as cephalothin, colistin, and polymyxin B, may be inhibitory to some strains of *C. jejuni* and *C. coli*, and are inhibitory to *C. fetus*.[1] 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 CVA Agar 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

Escherichia coli ATCC® 25922	B	48hr	35°C	Aerobic	No growth; partial to complete inhibition

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

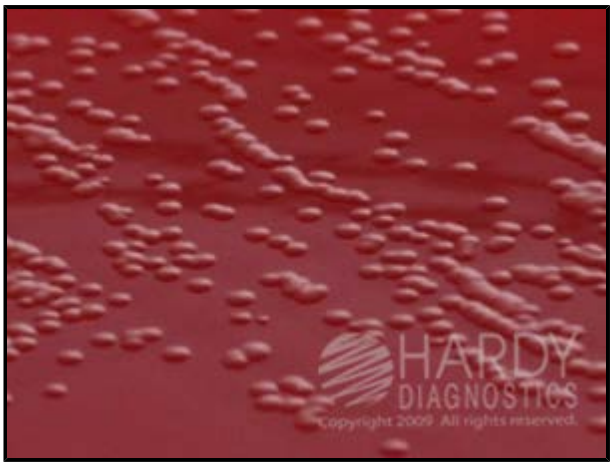
** Atmosphere of incubation is enriched with 5% O_2, 10% CO_2, and 85% N_2.

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 CVA Agar should appear opaque, and cherry red in color.



Campylobacter jejuni (ATCC® 33291) colonies growing on Campy CVA Agar (Cat. no. A40). Incubated under microaerophilic conditions for 48 hours at 35ºC.

Escherichia coli (ATCC® 25922) growth inhibited on Campy CVA Agar (Cat. no. A40). Incubated aerobically for 24 hours at 35ºC.

REFERENCES

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HDQA 2207F [D]