IFU

Instructions for Use

CAMPY, BLOOD-FREE AGAR

Cat. no. G06	Campy, Blood-Free Agar, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Campy, Blood-Free Agar is a selective medium recommended for the isolation and cultivation of *Campylobacter jejuni*, *C. coli*, and *C. laridis* from feces.[1]

SUMMARY

Campylobacter are a major enteric pathogen and a variety of culture media have been developed to isolate these thermophilic enteropathogenic microorganisms. There are a number of selective media for the isolation of *C. jejuni* and *C. coli*., including blood-free and blood containing formulations; however, formulations containing blood are disadvantagious for developing countries where supplies for sterile blood are not optimal.

To achieve the highest yield of *Campylobacter* organisms from stool, a combination of media may increase recovery rates by as much as 10 to 15% over the use of a single formulation.[5,7] In addition, the use of cefoperazone-containing media, as opposed to cephalothin-containing media, is recommended for the primary isolation of *Campylobacter* from fecal samples.[2,5,7] Specimens cultured on selective media should also be cultured on non-selective media to obtain additional information and to help ensure recovery of potential pathogens.

Campy, Blood-Free Agar is a charcoal based media supplemented with hematin and pyruvic acid, and made selective by the addition of cefoperazone, vancomycin and amphotericin B. The combination of a charcoal base, which neutralizes toxic oxygen derivatives, and the combined selectivity of the antimicrobics provides for an excellent media for the recovery of *C. jejuni*, *C. coli*, and *C. laridis* from feces.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	12.0gm
Peptic Digest of Animal Tissue	5.0gm
Sodium Chloride	5.0gm
Activated Charcoal	4.0gm
Yeast Extract	3.0gm
Beef Extract	3.0gm
Corn Starch	1.0gm
Pyruvic Acid	100.0mg

Hematin	32.0mg
Cefoperazone	20.0mg
Vancomycin	20.0mg
Amphotericin B	2.0mg
Agar	13.5gm

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), 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: Specimens 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, specimens should be inoculated into an appropriate transport media and refrigerated until inoculation.

Method of Use: Bring media to room temperature before use. Inoculate media with stool specimen using a sterile swab. Alternatively, drop 1-2 drops of liquid specimen using a sterile pipet. Streak to obtain isolated colonies. Incubate at 42ºC. in a microaerophilic atmosphere containing 5% O_2, 10% CO_2, and 85% N_2 for a minimum of 48 hours. Examine for growth and screen suspect colonies.

INTERPRETATION OF RESULTS

C. jejuni grows as a small colony, usually grayish and flat with irregular edges. A small percentage of strains may appear tan or slightly pinkish. Colonies tend to spread or swarm, especially when initially isolated from fresh clinical specimens.

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.

Additional tests including gram stain and biochemical testing should be performed on pure cultures for complete identification. For more information see appropriate references.[2-4]

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.[2]

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, swabs, applicator sticks, 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	24-48hr	35°C	Microaerophilic**	Growth	
Escherichia coli ATCC® 25922***	B	24hr	35°C	Aerobic		Partial to complete inhibition
Proteus mirabilis ATCC® 12453	B	24hr	35°C	Aerobic	Inhibited	

* 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.

*** 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 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, Blood-Free Agar should appear opaque, with slight traces of precipitate, and black in color.

Campylobacter jejuni (ATCC® 33291) colonies on Campy, Blood-Free Agar (Cat. no. G06). Incubated under microaerophilic conditions for 48 hours at 35ºC.

Escherichia coli (ATCC® 25922) growth inhibited on Campy, Blood-Free Agar (Cat no. G06). Incubated aerobically for 24 hours at 35ºC.

REFERENCES

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

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

5. Karmali, M.A., et al. 1986. *Evaluation of a blood-free, charcoal-based medium for the isolation of Campylobacter organisms from feces. J. Clin. Mircrobiol.*; 23:456-459.

6. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria,*, Lipincott Williams & Wilkins, Philadelphia, PA.

7. Oyarzabal, O.A., K.S. Macklin, J.M. Barbaree, and R.S. Miller. 2005. Evaluation of Agar Plates for Direct Enumeration of *Camplyobacter spp.* from Poultry Carcass Rinses. *Appl. and Environ. Microbio.* 71(6):3351-3354.

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

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