

CAMPYLOBACTER BLOOD AGAR

Cat. no. A137 Can	mpylobacter Blood Agar, 15x100mm plate, 18ml	10 plates/bag
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INTENDED USE

Hardy Diagnostics Campylobacter Blood Agar is recommended for the primary isolation and cultivation of *Campylobacter jejuni* from human fecal specimens.⁽¹⁻⁴⁾

SUMMARY

Dekeyser et al. reported the isolation of *Campylobacter jejuni* from the feces of patients with diarrhea and acute gastroenteritis by using a filtration technique and a selective medium with antimicrobials to suppress the normal enteric flora.⁽⁵⁾ In 1977, Skirrow reported a selective medium containing three antimicrobics.⁽⁶⁾ Blaser et al. in 1978, reported success in isolating *C. jejuni* with a medium containing four antimicrobics incorporated into Brucella Agar supplemented with 10% defibrinated sheep blood.^(1,2)

Peptones, dextrose, yeast extract, and blood support the growth of *Campylobacter* species. The peptones supply the nitrogen compounds, carbon, sulfur, and trace ingredients. Yeast extract is a source of B vitamins while dextrose is utilized as an energy source. Sheep blood supplies additional nutrients. The microbial agents, polymyxin B, trimethoprim, nystatin, and vancomycin, suppress the growth of the normal microbial flora in fecal specimens, thereby facilitating isolation of *C. jejuni*. Vancomycin inhibits gram-positive bacteria and polymyxin B inhibits most gramnegative bacilli except for *Proteus*. Trimethoprim is inhibitory for *Proteus* spp. Nystatin is an antifungal agent.

FORMULA

Ingredients per liter of deionized water:*

Agar	13.0gm
Trimethoprim	15.0gm
Pancreatic Digest of Casein	10.0gm
Pancreatic Digest of Animal Tissue	10.0gm
Sodium Chloride	5.0gm
Yeast Extract	3.0gm
Dextrose	1.0gm
Vancomycin	1.0gm
Nystatin	0.63gm
Polymyxin B	0.5gm

Sodium Bisulfite	0.1gm
Sheep Blood	100.0ml

Final pH 7.4 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. 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, moisture, 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 "<u>Storage</u>" 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 laboratory 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 "<u>Guidelines for Isolation</u> <u>Precautions</u>" 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 and handling.⁽⁸⁻¹¹⁾ Specimens should be obtained before antimicrobial therapy has been administered. 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, such as Campy Thioglycollate (Cat. no. K128) and refrigerate until inoculation.

When possible the clinical specimen should be inoculated directly onto the medium to prevent loss of organism viability. A liquid specimen may be directly applied to the agar surface and streaked with a sterile inoculating loop.

Incubate inoculated plates at 42°C. in an atmosphere conducive to the primary isolation and cultivation of microaerophilic organisms such as Cat. no. CN020C, or CN025A. Examine plates at 24 and 48 hours.

INTERPRETATION OF RESULTS

Campylobacter jejuni will appear as small, mucoid colonies, usually grayish in coloration, flat with irregular edges, and nonhemolytic at 24-48 hours.⁽⁸⁾ An alternate colonial morphology that appears to be strain related consists of round colonies 1-2mm in diameter that are convex, entire, and glistening.⁽⁸⁾ Colonies tend to spread or swarm, especially when initially isolated from fresh clinical specimens.

If the plates are to be examined at 24 hours, treat as if they were anaerobic cultures. Examine the plates quickly and place them back into a reduced oxygen atmosphere immediately after examination.

Consult listed references for further interpretation of growth.⁽⁸⁻¹¹⁾

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.

C. jejuni is thermophilic so it is important to incubate the plates at 42°C. If incubated at lower temperatures growth may be delayed and the selectivity of the medium is reduced.

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, anaerobe jars or bags, microaerophilic generators, applicator sticks, other culture media, 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		Incubation		Results
	Method*	Time	Temperature	Atmosphere	Kesuns
Campylobacter jejuni ATCC [®] 33291***	А	48hr	35°C	Micro**	Growth
Escherichia coli ATCC [®] 25922***	В	48hr	35°C	Aerobic	Partial to complete inhibition

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

** Microaerophillic atmospheric requirements, incubate in a jar with an appropriate atmospheric generator (Cat. no. CN025A).

*** 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 <u>Certificate of Analysis</u> website. Also refer to the document "<u>Finished Product</u>

<u>Quality Control Procedures</u>," 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

Campylobacter Blood Agar should appear opaque, and cherry red in color with no precipitate, chips or debris.

REFERENCES

1. Blaser, M., J. Cravens, B.W. Powers, and W.L. Wang. 1978. *Campylobacter enteritis* associated with canine infection. *Lancet* ii: 979-980.

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3. Reller, L.B., W-L.L. Wang, and M.J. Blaser. 1979. *Campylobacter enteritis* : *Campylobacter fetus* subspecies *jejuni* . ASPC Check Sample, Microbiology No. MB-99, Commission on Continuing Education, American Society of Clinical Pathologists, Chicago.

4. Smith, J.P., K. Durfee, and J.H. Marymont, Jr. 1980. Incidence of *Campylobacter enteritis* in the midwestern United States. *Am. J. Med. Technol.*; 46:81-84.

5. Dekeyser, P., M. Gossuin-Detrain, J.P. Butzler, and J. Sternon. 1972. Acute enteritis due to related *Vibrio* : first positive stool cultures. *J. infect. Dis.* ; 125:390-392.

6. Skirrow, M.B. 1977. Campylobacter enteritis : a "new" disease. Br. Med. J. 2: 9-11.

7. 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.

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

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

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

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

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

ATCC is a registered trademark of the American Type Culture Collection.

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