

Instructions for Use

BOLTON BROTH

Cat. no. U83	Bolton Broth, 500ml Polycarbonate Bottle, 225ml	10 bottles/box
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INTENDED USE

Hardy Diagnostics Bolton Broth is recommended for the selective enrichment of *Campylobacter* spp. from food and dairy products. This medium aids in the resuscitation of sublethally damaged cells of *Campylobacter*, while inhibiting the growth of undesirable gram-positive and gram-negative microorganisms, yeasts, and molds.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Campylobacter spp. are widely distributed in the environment and are a major cause of bacterial gastroenteritis. Many species commonly reside in the intestinal tract of a wide range of domestic and wild animals: cattle, sheep, pigs and birds. Consequently, foods derived from these animals can become contaminated with *Campylobacter* spp. when prepared under suboptimal conditions.^(5,6) Poultry is the most common source of bacterial gastroenteritis; however, cross-contamination to other foods prepared in common areas with infected meat products are also responsible for infection.⁽⁶⁾

Several species of campylobacters are thermotolerant and microaerophilic; these microorganisms can continue to grow optimally at temperatures up to 42°C. and prefer to live in oxygen-reduced environments. The most common strains of thermotolerant infectious campylobacters are *Campylobacter jejuni* and *Campylobacter coli*.^(5,6)

Cells of *Campylobacter* spp. can be difficult to culture as these bacteria may be infectious in very low numbers but are often found in the presence of a high number of competing microorganisms.⁽⁶⁾ More importantly, cells may become sublethally damaged during the processing of contaminated food products and, thus, are difficult to isolate. Traditional methods of culture and subculture have utilized selective enrichment medias, combined with the use of antibiotics to suppress the growth of competitors, and biochemical assays.⁽⁶⁾

Hardy Diagnostics Bolton Broth as an enrichment media was developed to optimize the growth of *Campylobacter* spp. from contaminated food and dairy products. This medium contains enzymatic digests of animal tissues, lactalbumin hydrolysates and yeast extract which provide essential nutrients like vitamins, amino acids and other nitrogenous compounds. Sodium metabisulphite and sodium pyruvate reduce toxic compounds and increase the recovery rate and aero-tolerance of the culture. Alpha-ketoglutaric acid provides a stimulus to metabolism, whereas sodium carbonate neutralizes acids that may form in the culture. The osmotic balance is further maintained by the addition of sodium chloride. The antibiotics vancomycin, cefoperazone and trimethoprim inhibit the growth of gram-positive and gram-negative microorganisms. Cycloheximide largely reduces the growth of yeast and molds. Finally, the incubation temperature of 41.5°C., after the initial incubation step, increases the selectivity of this medium making it easier to detect the presence of infectious *Campylobacter* spp.

FORMULA

Ingredients per liter of deionized water:*

Enzymatic Digest of Animal Tissues	10.0gm
Lactalbumin Hydrolysate	5.0gm
Yeast Extract	5.0gm
Sodium Chloride	5.0gm
α -Ketoglutaric Acid	1.0gm
Sodium Carbonate	0.6gm
Sodium Pyruvate	0.5gm
Sodium Metabisulfite	0.5gm
Hemin	0.01gm
Horse Blood, Lysed	50.0ml

Bolton Broth Supplement Solution	
Cycloheximide	50.0mg
Cefoperazone	20.0mg
Vancomycin	20.0mg
Trimethoprim	20.0mg

Final pH 7.4 +/- 0.3 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, 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 "[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 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 "[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

1. Mix 11.0gm of food sample into 100ml Bolton Broth or 25.0gm of food sample into 225ml of Bolton Broth and mix to combine thoroughly.
2. Incubate sample for 4 hours at 35°C. Continue incubation for an additional 14 to 44 hours at 42°C.
3. Subculture to appropriate isolation medium for further analysis. Consult listed references for more information.^(2-4,8,9)

INTERPRETATION OF RESULTS

Due to the selectivity of the medium and the elevated incubation temperature, growth upon subculture indicates a positive reaction; partial to complete inhibition upon subculture indicates a negative reaction.

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.

For identification, the organism must be in pure culture. Morphological, biochemical and /or serological tests should be performed for final identification. Consult appropriate references for detailed information and recommended procedures.^(2-4,8,9)

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, 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	
<i>Campylobacter jejuni</i> ATCC® 33291**	A	24-48hr	35°C	Micro	Growth; upon subculture
<i>Escherichia coli</i> ATCC® 25922**	B	24-48hr	35°C	Aerobic	Inhibited; partial to complete inhibition upon subculture

* 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 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

Bolton Broth should appear clear, and deep brick-red in color.

REFERENCES

1. Anderson, N.L., et al. 2005. *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. Krieg, et al. 1984. *Bergey's Manual of Systematic Bacteriology*, Vol. I. Williams & Wilkins Co., Baltimore, MD.
4. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
5. Heisick, J. 1985. Comparison of Enrichment Broths for Isoaltion of *Campylobacter jejuni*. *Appl. Environ. Microbiol.*; 50:1313-1314.
6. Baylis, C.L., S. MacPhee, K.W. Martin, T.J. Humphre, and R.P. Betts. 2000. Comparison of three enrichment media for the isolation of *Campylobacter* spp. from foods. *J. Appl. Microbiol.*; 89:884-891.
7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
8. American Public Health Association. *Standard Methods for the Examination of Dairy Products*, APHA, Washington, D.C.
9. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.
10. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*. AOAC, Arlington, VA.
<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm>

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IFU-10077[B]



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