

Instructions for Use

HUNT BROTH

Cat. no. U446	Hunt Broth, 125mL Plastic Bottle, 30ml	16 bottles/box
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INTENDED USE

Hardy Diagnostics Hunt Broth is recommended for the selective enrichment of *Campylobacter jejuni*, *C. coli*, and *C. lari* from poultry rinsate, poultry carcass samples, raw poultry samples, and/or environmental sponge samples.

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.⁽³⁻⁴⁾ 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.⁽⁴⁾

In comparison with other enrichment broths for recovery of *Campylobacter jejuni* from samples, Hunt broth exhibits the most prolific and rapid enrichment efficacy.⁽⁷⁾ Consequently, Hunt Broth is recommended for the isolation of *Campylobacter* by AOAC International, the American Public Health Association (APHA), and by the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS). The medium is designed as a selective enrichment broth for use with an equal volume of sample prepared in Buffered Peptone Water (e.g. [Cat. no. U143](#)).

The USDA FSIS MLG 41.07 outlines the use of Hunt Broth for the cultivation of control strains, as well as for selective enrichment of *Campylobacter* spp. from test samples. It is believed enrichment using Hunt Broth, followed by direct plating, can increase the sensitivity of *Campylobacter* qualitative detection methods for low levels of sub-lethally injured cells. After enrichment, presumptive positive cultures are subcultured to Campy Cefex Agar ([Cat. no. A122](#)) plates for isolation of strains for further testing and identification.

Hardy Diagnostics Hunt Broth is designed to comply with the USDA FSIS formulation, and contains peptones, yeast extract, and lysed horse blood, which act as sources of essential nutrients, amino acids, vitamins, minerals, and growth factors. Sodium pyruvate, sodium metabisulfite, and ferrous sulfate help maintain *Campylobacter* spp. characteristic morphology, motility, and aerotolerance viability by scavenging oxygen molecules from the medium. Sodium chloride aids in maintaining osmotic equilibrium. The selective ingredients, Amphotericin B, Cefoperazone, Vancomycin, and Trimethoprim, prevent growth of competing flora in heavily contaminated samples.

FORMULA

Ingredients per 950ml of deionized water:*

Beef extract	10.0g
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Peptone	10.0g
Yeast extract	6.0g
Sodium chloride	5.0g
Ferrous sulfate	0.25g
Sodium pyruvate	0.25g
Sodium metabisulfite	0.25g
Amphotericin B	20.0mg
Cefoperazone	30.0mg
Vancomycin	10.0mg
Trimethoprim	12.5mg
Lysed horse blood	50.0ml

Final pH 7.0 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt, store away from direct light at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration, discoloration, 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

Sample Collection: Consult reference methods for information on sample collection.⁽¹⁻²⁾ Samples should be submitted directly to the laboratory without delay and protected from excessive heat and cold.

Method of Use: Allow the medium to warm to room temperature prior to inoculation. Consult references for information concerning inoculation procedures.⁽¹⁻²⁾

1. Combine an equal volume of sample prepared in Buffered Peptone Water (BPW) with an equal volume of Hunt Broth (e.g. 30ml sample to 30ml Hunt Broth) in a vented culture flask, Whirl-Pak® bag, or equivalent.
2. Gently combine the sample and broth together to ensure an even distribution.
3. Incubate the inoculated broth for 22 to 26 hours at 41-43°C using microaerobic conditions.
4. Following incubation, follow the USDA FSIS MLG 41.07 procedure for performing a rapid screen test. For rapid screen positive cultures, use a four quadrant streak to isolate colonies from presumptive positive samples to Campy Cefex Agar ([Cat. no. A122](#)) using a sterile, non-metal, 10µl loop.
5. Incubate Campy Cefex Agar at 41-43°C for 46 to 50 hours using microaerobic conditions.
6. Examine plates for isolated colonies showing typical colony morphology characteristic of *Campylobacter* spp. and perform confirmatory identification test(s), as needed.

INTERPRETATION OF RESULTS

Consult listed references for appropriate interpretation of results.⁽¹⁻²⁾ After enrichment broth procedures and rapid screen tests, examine incubated Campy Cefex Agar ([Cat. no. A122](#)) plates for typical colonies that are translucent or mucoid, glistening and pink in color, flat or slightly raised. Colonies may vary in size. A reddish halo may also be present in the surrounding agar. Confirm isolates using MALDI-TOF (Cat. no. MS1000) or other confirmatory test method(s).

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.

Hunt Broth is designed to be combined with an equal volume of sample prepared in Buffered Peptone Water ([e.g. Cat. no. U143](#)) or similar rinsate solution during the enrichment step. After Hunt Broth enrichment incubation, a rapid screen test should be performed, and presumptive positive broths should be subcultured to Campy Cefex Agar ([Cat. no. A122](#)). Isolates from Campy Cefex Agar should be confirmed using validated identification method(s).

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 such as Buffered Peptone Water ([Cat. no. U143](#)) and Campy Cefex Agar ([Cat. no. A122](#)), rapid screening tools or identification methods such as MALDI-TOF ([Cat. no. MS1000](#)) and associated reagents, incinerators, 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	46-50hr	41-43°C	Microaerophilic**	Growth upon subculture***
<i>Campylobacter coli</i> ATCC® 33559	A	46-50hr	41-43°C	Microaerophilic**	Growth upon subculture***
<i>Campylobacter lari</i> ATCC® 35221	A	46-50hr	41-43°C	Microaerophilic**	Growth upon subculture***
<i>Escherichia coli</i> ATCC® 25922	B	24-48hr	35°C	Aerobic	Partial to complete inhibition upon subculture***
<i>Candida albicans</i> ATCC® 10231	B	24-48hr	35°C	Aerobic	Partial to complete inhibition upon subculture***

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

** Atmosphere of incubation is enriched with 5% O₂, 10% CO₂, and 85% N₂.

*** Hunt Broth is combined with organism in equal volume of BPW and incubated under microaerobic conditions for 22-26 hours at 41-43°C for *Campylobacter* strains, and aerobically at 35°C for *E. coli* and *C. albicans*. Broth is then subcultured to Campy-Cefex (Cat. no. A122) or Campy CVA (Cat.no.A40) to confirm performance characteristics.

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

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

REFERENCES

1. United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS). Microbiology Laboratory Guidebook (MLG) [41.07 Isolating and Identifying Campylobacter jejuni/coli/lari from Poultry Rinsate, Sponge, and Raw Product Samples](#). March 07, 2022. USDA/FSIS/OPHS Athens, GA.
2. United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS). Microbiology Laboratory Guidebook (MLG) Appendix 2.04. [FSIS Laboratory Flow Chart for Campylobacter jejuni/coli/lari Enrichment Analysis](#). March 07, 2022. USDA/FSIS/OPHS Athens, GA.
3. Heisick, J. 1985. Comparison of Enrichment Broths for Isolation of *Campylobacter jejuni*. *Appl. Environ. Microbiol.*; 50:1313-1314.
4. 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.
5. American Public Health Association. *Standard Methods for the Examination of Dairy Products*, APHA, Washington, D.C.

6. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.

7. Kim, S.A., Y.M. Lee, I.G. Hwang, D.H. Kang, G.J.Woo, and M.S. Rhee. Eight enrichment broths for the isolation of *Campylobacter jejuni* from inoculated suspensions and ground pork. *Letters in Appl. Microbio.* 49(5): 620-626.

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

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