Campylobacter Enrichment Broth (Bolton Broth)
SKU: 700003230, 700003231, 700003232, 700003233, 700003234
NCM0094

Intended Use
Campylobacter Enrichment Broth (Bolton Broth) is used with antimicrobics for the selective enrichment of *Campylobacter* spp according to ISO 10272-1:2017 and USDA-MLG. Campylobacter Enrichment Broth (Bolton Broth) is not intended for use in the diagnosis of disease or other conditions in humans.

Description
Campylobacter spp. are microaerophilic, very small, curved, thin, Gram-negative rods. Microaerophilic organisms have a tendency to be more sensitive to toxic forms of oxygen. Campylobacter Enrichment Broth (Bolton), along with nutritional ingredients, contains compounds which enhance the aerotolerance of microaerophilic bacteria by suppressing the toxic form of oxygen. Campylobacter Enrichment Broth (Bolton) is recommended in food testing. Blood-Free Campylobacter Enrichment Broth, Bolton Broth (2X Concentration) is described by the USDA.

Enzymatic Digest of Animal Tissue, Lactalbumin Hydrolysate, and Yeast Extract provide nitrogen, carbon, amino acids, and vitamins in Campylobacter Enrichment Broth. Hemin and Lysed Horse Blood provide essential growth factors. Sodium Chloride maintains the osmotic balance of the medium. Sodium Pyruvate, Sodium Metabisulfite, and Sodium Carbonate increase the aerotolerance of *Campylobacter* spp. by acting as oxygen scavengers. The addition of cefoperazone, amphotericin, trimethoprim, and vancomycin are selective agents for heavily contaminated samples.

Typical Formulation

Enzymatic Digest of Animal Tissue	10.0 g/L
Lactalbumin Hydrolysate	5.0 g/L
Yeast Extract	5.0 g/L
Sodium Chloride	5.0 g/L
Hemin	0.01 g/L
Sodium Pyruvate	0.5 g/L
α-Ketoglutaric Acid	1.0 g/L
Sodium Metabisulfite	0.5 g/L
Sodium Carbonate	0.6 g/L

Final pH: 7.4 ± 0.2 at 25°C
Formula is adjusted and/or supplemented as required to meet performance specifications.

Supplements
NCM4074 Campylobacter Bolton (with Amphotericin)

Precaution
Refer to SDS

620 Lesher Place • Lansing, MI 48912
800-234-5333 (USA/Canada) • 517-372-9200
foodsafety@neogen.com • foodsafety.neogen.com

Preparation

1. Dissolve 27.6 grams of the medium in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium, if necessary.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and aseptically add 50 mL of lysed horse blood and 2 vials of 700004915* Campylobacter Bolton (with Amphotericin) each reconstituted using 5 mL sterile 50% ethanol.
5. Note: Blood-Free Campylobacter Enrichment Broth, Bolton's (2X Concentration) is described by the USDA.

*Larger vials may be available. Please see appropriate supplement data sheet for availability and preparation instructions.

Test Procedure

Refer to the appropriate references for the material being tested regarding the isolation of *Campylobacter* spp. If using the ISO method, refer to ISO 10272-1:2017.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige to beige.

Prepared Appearance (Un-supplemented): Prepared medium is clear to trace hazy, amber to dark amber, and may have none to light precipitate with fine black particles.

Prepared Appearance (Supplemented): Prepared medium is amber to dark amber to dark red-amber, with none to moderate precipitate.

Expected Cultural Response: The medium was prepared according to label directions and inoculated with the organisms listed below. Cultures were incubated for 5 ± 1 hours in a microaerophilic atmosphere at 37 ± 1°C in the Campylobacter Enrichment Broth. Then, transferred to incubate for 44 ± 4hrs at 41.5 ± 1°C. The Campylobacter Enrichment Broth was then examined for confirmation of recovery or inhibition by subculture onto non-selective blood agar media.

Microorganism	Approx. Inoculum (CFU)	Expected Growth
Campylobacter jejuni ATCC® 29428	10 – 100	Growth
Campylobacter jejuni ATCC® 33291	10 – 100	Growth
Campylobacter coli ATCC® 43478	10 – 100	Growth
Campylobacter lari ATCC® 35221	10 – 100	Growth
Enterococcus faecalis ATCC® 29212	>1000	Inhibited
Escherichia coli ATCC® 8739	>1000	Inhibited
Proteus mirabilis ATCC® 29906	>1000	Inhibited

The organisms listed tare the minimum that should be used for quality control testing.

Results

Campylobacter colonies are round to irregular with smooth edges. They may have translucent, white colonies to spreading, flat, transparent growth. Some strains appear tan or slightly pink. Normal enteric flora are completely to markedly inhibited. Typically, *Campylobacter* spp. are oxidase positive and catalase positive. For complete identification of species and biotype, refer to the appropriate procedures for biochemical reactions.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Denatured ethanol must not be used because the additives could possibly be toxic to *Campylobacter*.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm.
2. George, H. A., P. S. Hoffman, and N. R. Krieg. 1978. J. Clin. Micro. 8:36-41.
3. United States of Agriculture Food Safety and Inspection Service. 2011. Microbiology Laboratory Guidebook, Appendix 1.05. Athens, Georgia.
4. United States Department of Agriculture, Food Safety and Inspection Service, 2010. Isolation, identification, and enumeration of *Campylobacter jejuni/coli/lari* from poultry rinse and sponge samples. MLG 41.00, USDA/FSIS, Microbiology Laboratory Guidebook, Washington D.C.
5. Murray, P. R., E. J. Baron, M. A. Pfaller, J. A. Jorgensen, M. L. Landry (eds.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
6. ISO 10272-1:2017 Microbiology of the food chain – Horizontal method for detection and enumeration of Campylobacter spp. – Part 1: Detection method

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