CAMPYLOBACTER ENRICHMENT BROTH (BOLTON’S) (7526)

**Intended Use**
Campylobacter Enrichment Broth (Bolton's) is used with antimicrobics for the selective enrichment of Campylobacter spp in a laboratory setting. Campylobacter Enrichment Broth (Bolton's) is not intended for use in the diagnosis of disease or other conditions in humans.

**Product Summary and Explanation**
Campylobacter spp. are microaerophilic, very small, curved, thin, Gram-negative rods.¹ Microaerophilic organisms have a tendency to be more sensitive to toxic forms of oxygen.² Campylobacter Enrichment Broth (Bolton’s), along with nutritional ingredients, contains compounds which enhance the aerotolerance of microaerophilic bacteria by suppressing the toxic form of oxygen.² Campylobacter Enrichment Broth (Bolton’s) is recommended in food testing.¹ Blood-Free Campylobacter Enrichment Broth, Bolton’s (2X Concentration) is described by the USDA.³

**Principles of the Procedure**
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 Metabisulphite, and Sodium Carbonate increase the aerotolerance of Campylobacter spp. by acting as oxygen scavengers. The addition of cefoperazone, cycloheximide, trimethoprim, and vancomycin are selective agents for heavily contaminated samples.

**Formula / Liter**
Enzymatic Digest of Animal Tissue .......................................................... 10 g
Lactalbumin Hydrolysate ............................................................................. 5 g
Yeast Extract ............................................................................................... 5 g
Sodium Chloride ......................................................................................... 5 g
Hemin ........................................................................................................... 0.01 g
Sodium Pyruvate .......................................................................................... 0.5 g
α-Ketoglutaric Acid ....................................................................................... 1 g
Sodium Metabisulfite ................................................................................... 0.5 g
Sodium Carbonate ....................................................................................... 0.6 g
Final pH: 7.4 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

**Campylobacter Supplement (Bolton), 5 mL (# 7998)**
2.5 ml ethanol / 2.5 mL purified water

**Enrichment**
Lysed Horse Blood 50 mL

**Antimicrobic / 10 mL of Ethanol**
- Cefoperazone 20 mg
- Cycloheximide 50 mg
- Trimethoprim 20 mg
- Vancomycin 20 mg

**Precautions**
1. For Laboratory Use.
2. HARMFUL. Harmful if swallowed, inhaled, or absorbed through skin. May cause allergic respiratory reaction. Irritating to eyes, skin, and respiratory tract.
Directions
1. Dissolve 27.6 g of the medium in one liter of purified water.
2. Allow powder to soak for 10 minutes.
3. Heat with frequent agitation to completely dissolve the medium.
4. Autoclave at 121°C for 15 minutes.
5. Cool medium to 45 - 50°C and if desired, aseptically add 50 mL of lysed horse blood and 2 vials of reconstituted Campylobacter Supplement (Bolton's) (# 7998), OR 10 mL of ethanol (USP grade recommended) containing 20 mg of Cefoperazone, 50 mg of Cycloheximide, 20 mg Trimethoprim, and 20 mg Vancomycin.
6. Campylobacter Supplement (Bolton's) (# 7998) is aseptically reconstituted by adding 2.5 mL ethanol (USP grade) and 2.5 mL of sterile purified water to the vial. Gently swirl to dissolve the contents.
7. Note: Blood-Free Campylobacter Enrichment Broth, Bolton's (2X Concentration) is described by the USDA.

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

Prepared Appearance (Unsupplemented): 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: Cultural response, after incubation in Campylobacter Enrichment Broth (Bolton's) for 24 – 48 hours in a microaerophilic atmosphere at 42 ± 1°C. Cultures were then examined for confirmation of recovery or inhibition by subculturing onto non-selective blood agar media.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Approx. Inoculum (CFU)</th>
<th>Expected Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter jejuni ATCC® 29428</td>
<td>10 - 300</td>
<td>Growth</td>
</tr>
<tr>
<td>Campylobacter coli ATCC® 33559</td>
<td>10 - 300</td>
<td>Growth</td>
</tr>
<tr>
<td>Campylobacter lari ATCC® 35221</td>
<td>10 - 300</td>
<td>Growth</td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC® 29212</td>
<td>1000</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Escherichia coli ATCC® 25922</td>
<td>1000</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Proteus mirabilis ATCC® 12453</td>
<td>1000</td>
<td>Inhibited</td>
</tr>
</tbody>
</table>

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

Test Procedure
Refer to the appropriate procedure for the material being testing on the isolation of Campylobacter spp. Refer to appropriate references for Campylobacter 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.

Storage
Store dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.
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.³

Packaging
Campylobacter Enrichment Broth (Bolton’s)  
Code No.  
7526A  500 g  
7526B  2 kg  
7526C  10 kg  

Campylobacter Supplement (Bolton), 5 mL  
Code No.  7998

References

Technical Information
Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.