Bolton Broth Base

M1592

Bolton Broth Base is used for the selective enrichment of *Campylobacter* species from foods.

Composition**

Ingredients	Gms / Litre
Enzymatic digest of animal tissues	10.000
Lactalbumin hydrolysate	5.000
Yeast extract	5.000
Sodium chloride	5.000
Sodium metabisulphite	0.500
Sodium carbonate	0.600
Hemin	0.010
alpha-ketoglutaric Acid	1.000
Sodium pyruvate	0.500
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 13.8 grams in 500 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add rehydrated contents of 1 vial of Bolton Selective supplement (FD231) and 25 ml of sterile lysed defibrinated horse blood in the medium. Horse blood may be saponin lysed or lysed by freezing then thawing out. Mix well and aseptically dispense into sterile tubes.

Principle And Interpretation

Foods of animal origin are the primary vehicles of *Campylobacter* infections in humans. Unpasteurized milk has been by far the most commonly implicated vehicle in the foodborne outbreaks of *Campylobacter jejuni* enteritis (1, 2). *Campylobacters* were originally classified within the genus *Vibrio* , but they differ from *Vibrios* in their DNA Base composition and their ability to grow under conditions of reduced oxygen tension. Selective media were originally designed to isolate *Campylobacter jejuni* from faeces, by use of a cocktail of antibiotics in a rich basal medium (3). Bolton Broth Base is formulated as per recommendations of ISO for the selective enrichment of *Campylobacter* species from foods (4, 5, 6). The media is made selective for *Campylobacters* by addition of the antibiotics cefoperazone, vancomycin, trimethoprim and amphotericin B. These antibiotics are added as freeze dried supplements (FD).

The medium contains nutrients, which aid resuscitation of sublethally damaged cells of *Campylobacter* . Hence microaerophilic incubation is not needed. The supplement added to the medium contains four different antibiotics. Vancomycin, cefoperazone and trimethoprim inhibit the growth of gram-positive and gram-negative bacteria while amphotericin B largely reduces the growth of yeasts and moulds.

Inoculate a small quantity of the test sample into nine times its volume of Bolton Broth (M1592), so as to obtain a test portion/ enrichment medium ratio of 1:10 (mass/volume or volume/volume), and homogenize. Incubate at 37°C for 4-6 hours, then at 41.5°C for 44 ± 4 hours. A loopful of this enriched culture is streaked on Karmali Campylobacter Agar Base (M1222).

Quality Control

Appearance

Light yellow to brownish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Basal medium : Brownish yellow coloured clear to slightly opalescent solution. After addition of lysed horse blood: Red to brown coloured opaque solution in tubes.

Reaction

Reaction of 2.76% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
M1592: Cultural characteristics observed with added Bolton Selective Supplement (FD231) after an incubation at 35-37°C for 4-6 hours and then at 41.5°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth
Cultural Response		
Candida albicans ATCC 10231	>=10^3	inhibited
Campylobacter coli ATCC 33559	50-100	good-luxuriant
Campylobacter jejuni ATCC 29428	50-100	good-luxuriant
Escherichia coli ATCC 25922	>=10^3	inhibited

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1. Blasser M.J., Cravens J., Powers B.W., LaForce F.M., and Wang W. L.L., 1979, Am. J. Med., 67:715.
2. Brieseman M.A., 1984, N.Z. Med. J., 97:411.
3. Corry, Curtis and Baird. Culture Media For Food Microbiology, Vol.34. Progress in Industrial Microbiology, 1995, Elsevier, Amsterdam.
4. Hunt J.M, Campylobacter, F.D.A Bacteriological Analytical Manual, 8th Edition (Revision AOAC, Arlington V A (1998).
5. Bolton F. J., Personal communication (1995).
6. International Organization for Standardization (ISO), 2006, Draft ISO 10272- 1:2006 (E).

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