

Bolton Broth Selective Supplement

PRODUCT INFORMATION

C010-1g - Cefoperazone Sodium, Powder, 1g

C010-5g - Cefoperazone Sodium, Powder, 5g

V001-250mg - Vancomycin HCl, Powder, 250mg

V001-1g - Vancomycin HCl, Powder, 1g

V001-5g - Vancomycin HCl, Powder, 5g

T011-5g - Trimethoprim, Powder, 5g

T011-25g - Trimethoprim, Powder, 25g

T011-100g - Trimethoprim, Powder, 100g

C001-1g - Cycloheximide, Powder, 1g

C001-5g - Cycloheximide, Powder, 5g

DESCRIPTION

Bolton Broth with Bolton Broth Selective Supplement (Bolton Selective Enrichment Broth) is a medium for the selective pre-enrichment of *Campylobacter* organisms in food samples.

BACKGROUND

Cefoperazone is a third generation cephalosporin antibiotic. It is one of few cephalosporin antibiotics effective in treating *Pseudomonas* bacterial infections which are otherwise resistant to these antibiotics.

Vancomycin is a glycopeptide antibiotic used in the prophylaxis and treatment of infections caused by Gram-positive bacteria.

Trimethoprim is a bacteriostatic antibiotic which belongs to the class of chemotherapeutic agents known as dihydrofolate reductase inhibitors.

Cycloheximide is widely used in biomedical research to inhibit protein synthesis in eukaryotic cells studied in vitro (i.e. outside of organisms). Its effects are rapidly reversed by simply removing it from the culture medium.

Mechanism of action

Vancomycin acts by inhibiting proper cell wall synthesis in Gram-positive bacteria. Due to the different mechanism by which Gram-negative bacteria produce their cell walls and the various factors related to entering the outer membrane of Gram-negative organisms, vancomycin is not active against Gram-negative bacteria (except some non-gonococcal species of *Neisseria*).

Trimethoprim acts by interfering with the action of bacterial dihydrofolate reductase, inhibiting synthesis of tetrahydrofolic acid. Tetrahydrofolic acid is an essential precursor in the de novo synthesis of the intermediate Thymidine monophosphate (dTMP), precursor of DNA metabolite Thymidine triphosphate. Bacteria are unable to take up folic acid from the environment (i.e. the infection host) and are thus dependent on their own de novo synthesis. Inhibition of the enzyme starves the bacteria of nucleotides necessary for DNA replication causing, in certain circumstances, cell lethality due to thymineless death.

Cycloheximide is an inhibitor of protein biosynthesis in eukaryotic organisms, produced by the bacterium *Streptomyces griseus*. Cycloheximide exerts its effect by interfering with the translocation step in protein synthesis (movement of two tRNA molecules and mRNA in relation to the ribosome) thus blocking translational elongation.

APPLICATION IN BOLTON BROTH

Bolton Selective Enrichment Broth is intended for the pre-enrichment of *Campylobacter* in food samples. *Campylobacter* are Gram-negative, spirally shaped microaerophilic organisms which may be present in raw milk, untreated water, improperly handled food and undercooked meats, poultry and shellfish. Human consumption of these organisms can result in a range of clinical illnesses from transient asymptomatic colonisation to severe dysentery. The symptoms of *Campylobacter* enteritis include diarrhoea, stomach pain, nausea, fever, headache and muscle pain. Complications of infection by *Campylobacter jejuni* may include unnecessary appendectomies as a result of abdominal pain, reactive arthritis or Guillian-Barré syndrome¹. *Campylobacter* infection is recognised as one of the most common causes of bacterial gastro-

enteritis in humans, and the minimum infective dose may be as low as 500-800 cells.

Since awareness of the apparent role of *Campylobacter* in human disease was heightened by Skirrow in 1977, a great number of culture media have evolved in response to the need to optimise performance. There was early recognition of the need for enrichment culture when examining food samples to overcome the damaging effects that food processing and preservation techniques can have on *Campylobacter* cells. Use of lower incubation temperatures in the early stages of enrichment is now widely established as an aid to cell recovery. This principle was employed by Bolton in the development of his enrichment broth.

Campylobacter can be injured by food processing and preservation procedures. This makes them susceptible to selective agents which are tolerated by undamaged cells. False negative results are avoided through use of recovery medium such as Bolton Selective Enrichment Broth which increases the number of cells available for culture, first by resuscitating injured organisms and then encouraging them to multiply.

Bolton Selective Enrichment Broth contains nutrients to aid resuscitation of sublethally injured cells, and is formulated to avoid the need for a microaerobic atmosphere. Initial incubation is carried out at 30-37°C, depending on the type of food to be examined. After the pre-enrichment, the incubation temperature is raised to 42°C to increase the selective pressures on competing organisms.

Inclusion of sodium metabisulphite and sodium pyruvate in Bolton Broth quenches toxic compounds that may form in the culture medium. These additions also increase the aero-tolerance of the culture. The antibiotics contained in Bolton Broth Selective Supplement optimise selectivity for *Campylobacter* spp. Vancomycin - active against Gram-positives. Cefoperazone - predominantly active against Gram-negatives. Trimethoprim - active against a wide variety of Gram-negative and Gram-positive organisms. Cycloheximide - active against yeasts.

Content concentrations

Typical Formula*	mg/litre
Bolton Broth	
Meat peptone	10
Lactalbumin hydrolysate	5
Yeast Extract	5
Sodium chloride	5
Alpha-ketoglutaric acid	1

Sodium pyruvate	0.5
Sodium metabisulphite	0.5
Sodium carbonate	0.6
Haemin	0.01
Final pH 7.4 ± 0.2 @ 25°C	
Bolton Broth Selective Supplement	
Cefoperazone	20
Vancomycin	20
Trimethoprim	20
Cycloheximide	50
* Adjusted as required to meet performance standards	
Table 1 - Typical Formula for Bolton Broth and Bolton Broth Selective Supplement	

METHOD

Preparation

Add appropriate amount of Bolton Broth to distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 25 ml laked horse blood and Bolton Broth Selective Supplement, reconstituted as directed. Mix well and distribute into sterile screw top containers.

Protocol

One method of use is as follows:

- Place 25 g of food sample in 225 ml Bolton Selective Enrichment Broth (prepared as described above) and homogenise the mixture using a Stomacher (or similar device).
- Bolton Selective Enrichment Broth does not require incubation in a microaerobic environment, but must be used in screw topped containers which are filled to within 20 mm of the top. Incubate for 4 hours at 37°C, followed by further incubation at 42°C.
- The broth can be subcultured after 24 hours and 48 hours on to either Modified CCDA or Preston Agar.

For other methods please refer to BAM.

Quality control

Positive control:

Campylobacter jejuni ATCC® 29428: Good growth; grey coloured colonies

Negative control:

Escherichia coli ATCC® 25922: No growth

REFERENCES

1. National Advisory Committee on Microbiological Criteria for Foods (1994). *Journal of Food Protection* 57 (12): 1101-1121.
2. Skirrow, M.B. (1977). *British Medical Journal* 2: 9-11.
3. Post, D. E. (1995). *Food-Borne Pathogens Monograph Number 3 Campylobacter* .
4. Bolton, F.J. (1995) Personal communication.
5. Hunt, J.M. (1998) *Campylobacter*. In: F.D.A. *Bacteriological Analytical Manual*, 8th Edition (Revision A) 7.01-7.27. AOAC, Arlington Va.

