Modified Bolton Broth Selective Supplement

PRODUCT INFORMATION

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

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

T011-5g - Trimethoprim, Powder, 5g

T011-25g - Trimethoprim, Powder, 25g

T011-100g - Trimethoprim, Powder, 100g

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

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

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

A007-100mg - Amphotericin B, Powder, 100mg

A007-250mg - Amphotericin B, Powder, 250mg

A007-1g - Amphotericin B, Powder, 1g

A007-5g - Amphotericin B, Powder, 5g

DESCRIPTION

Bolton Broth with Modified Bolton Broth Selective Supplement (Modified 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.

Amphotericin B is a polyene antifungal drug, often used intravenously for systemic fungal infections. It was originally extracted from *Streptomyces nodosus*. Its name originates from the chemical's amphoteric properties. Two amphotericins, amphotericin A and amphotericin B are known, but only B is used clinically, because it is significantly more active in vivo.

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.

As with other polyene antifungals, amphotericin B associates with ergosterol, the main component of fungal cell membranes, forming a transmembrane channel that leads to monovalent ion (K+, Na+, H+ and Cl–) leakage, which is the primary effect leading to fungal cell death.

APPLICATION IN BOLTON BROTH

Modified 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 Modified Bolton Selective Enrichment Broth which increases the number of cells available for culture, first by resuscitating injured organisms and then encouraging them to multiply.

Modified 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 Modified 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. Amphotericin B - active against yeasts and fungal.

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	
Modified Bolton Broth Selective Supplement	
Cefoperazone	10
Trimethoprim	10
Vancomycin	10
Amphotericin B	5
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Bolton Broth and Modified 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 Modified Bolton Broth Selective Supplement, re-constituted as directed. Mix well and distribute into sterile screw top containers.

Protocol

One method of use is as follows:

1. Place 25 g of food sample in 225 ml Modified Bolton Selective Enrichment Broth (prepared as described above) and homogenise the mixture using a Stomacher (or similar device).

2. Modified 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.

3. 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.