

# VCC Selective Supplement

## PRODUCT INFORMATION

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

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

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

C051-25mg - Cefixime, Powder, 25mg

C051-100mg - Cefixime, Powder, 100mg

C058-100mg - Cefsulodin Sodium, Powder, 100 mg

C058-250mg - Cefsulodin Sodium, Powder, 250 mg

C058-1g - Cefsulodin Sodium, Powder, 1g

## DESCRIPTION

Modified Tryptone Soya Broth (mTSB) with VCC Selective Supplement is a medium designed for the selective enrichment of *Escherichia coli* O157 from food or faecal samples

## BACKGROUND

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

Cefixime is an oral third generation cephalosporin antibiotic.

Cefsulodin is a third generation cephalosporin antibiotic that has very specific activity against *Pseudomonas aeruginosa*. It has no significant activity against other Gram-negative bacteria and very limited activity against Gram-positive bacteria and anaerobic bacteria.

## 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*).

## APPLICATION IN MODIFIED TRYPTONE SOYA BROTH (MTSB)

Many selective broths have been formulated for the enrichment of *Escherichia coli* O157. mTSB is a nutritious, selective base, which was devised by Doyle and Shoeni. The broth was incubated for at 37°C for 24 hours in shaken flasks. Szabo and co-workers also used mTSB and found incubation at 43°C with agitation gave better recovery rates from inoculated food samples.

Buffered peptone water (BPW) supplemented with VCC Selective Supplement was used for the isolation of *Escherichia coli* O157 from beef carcasses, bovine faeces and from milk and minced beef.

In EHEC enrichment broth (EEB), VCC Selective Supplement is added to mTSB, which is used instead of BPW to overcome some of the inhibitory effects of the antibiotics on *Escherichia coli* O1574.

Both BPW-VCC and EEB are incubated for 6 hr at 37°C.

Tryptone soya broth has been modified by increasing the concentration of the buffer, dipotassium hydrogen phosphate, to 4 g/l and the addition of 1.5 g/l Bile Salts. The bile salts inhibit the growth of spore formers and enterococci, but allow the growth of *Escherichia coli* O157.

VCC Selective Supplement contains three antibiotics vancomycin for inhibition of Gram-positive organisms. Cefixime for the inhibition of *Proteus* spp. cefsulodin suppresses *Pseudomonas* spp.

## Content concentrations

Typical Formula*	mg/litre
<b>Modified Tryptone Soya Broth (mTSB)</b>	
Pancreatic digest of casein	17
Papaic digest of soybean meal	3
Sodium chloride	5
Dipotassium hydrogen phosphate	4
Glucose	2.5
Bile Salts	1.5
Final pH 7.4 ± 0.2 @ 25°C	
<b>VCC Selective Supplement</b>	
<a href="#">Vancomycin</a>	8.0

<a href="#">Cefixime</a>	0.05
<a href="#">Cefsulodin</a>	10
* Adjusted as required to meet performance standards	

**Table 1 - Typical Formula for Modified Tryptone Soya Broth (mTSB) and VCC Selective Supplement**

## METHOD

### Preparation

Suspend appropriate amount of Modified Tryptone Soya Broth (mTSB) in distilled water. Mix well, dispense into final containers and sterilise by autoclaving at 121°C for 15 minutes. Allow the medium to cool to 50°C before adding a selective supplement as appropriate.

### Protocol

mTSB can be used with either Novobiocin Selective Supplement or VCC Selective Supplement depending on which protocol is followed. VCC Selective Supplement is a highly selective supplement, which can be used either with mTSB, to give EHEC Enrichment Broth (EEB), or with Buffered Peptone Water depending on which protocol is followed.

#### 1. ISO5/ PHLS6/ FDA/BAM7/NMKL

mTSB+n

500ml of Modified Tryptone Soya Broth and 1 vial of Novobiocin Selective Supplement. Incubate at 41.5°C for 6 and 22 hours.

#### 2. FDA/BAM

EEB

225ml of Modified Tryptone Soya Broth and 1 vial of VCC Selective Supplement Incubate at 37°C with agitation for 6 and 24 hours.

#### 3. CCFRA

BPW-VCC

225ml of Buffered Peptone Water and VCC Selective Supplement. Incubate at 37°C for 6 and 24 hours.

### Quality control

Positive control:

*Escherichia coli* 0157:H7 NCTC 12900: Turbid growth

Negative control:

*Staphylococcus aureus* ATCC® 25923: Inhibited or no growth

## REFERENCES

1. Doyle, M. P. and J. L. Schoeni (1987) Applied Environmental Microbiology 53: 2394-2396
2. Szabo R. E. et al. (1990) Applied Environmental Microbiology 56: 3546-3549
3. Desmarchelier, P. M. and Grau, F. H. (1997) *Escherichia coli*. In: Foodborne Microorganisms of Public Health Significance. 5th Edition. pp.231-264. A. D. Hocking (Ed.). AIFST (NSW Branch) Food Microbiology Group, Australia
4. Weagant S. D. et al. (1995) Journal of Food Protection 58: 7-12
5. ISO:16654:2001 Microbiology of food and animal feed stuffs. Horizontal method for the detection of *E. coli* O157 (Pub March 22, 1999, expired June 30 1999)
6. PHLS Standard Methods F17 & W16: Detection of *E. coli* O157
7. FDA/BAM 8th Edition (Revision A) (1998) Chapter 4: 4.20-4.26
8. Campden and Chorleywood Food Research Association Method 19.1: 1997. Detection of *E. coli* O157
9. Nordic Committee on Food Analysis No 164 1999 *Escherichia coli* O157. Detection in food and feeding stuffs