Vancomycin Resistant Enterococci (VRE) Selective Supplement

PRODUCT INFORMATION

M002-10mg - Meropenem, Powder, 10mg

M002-50mg - Meropenem, Powder, 50mg

G006-1g - Gentamicin Sulfate, Powder, 1g

G006-5g - Gentamicin Sulfate, Powder, 5g

G006-25g - Gentamicin Sulfate, Powder, 25g

G035-10mg - Gentamicin A Sulfate, EvoPure™, 10mg

G031-10mg - Gentamicin C1 Sulfate, EvoPure™, 10mg

G032-10mg - Gentamicin C1a Sulfate, EvoPure™, 10mg

G033-10mg - Gentamicin C2 Sulfate, EvoPure™, 10mg

G034-10mg - Gentamicin C2a Sulfate, EvoPure™, 10mg

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

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

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

DESCRIPTION

VRE Broth Base and VRE Agar Base areselective media for the isolation of Vancomycin Resistant Enterococci (VRE) and High Level Aminoglycoside Resistant Enterococci (HLARE) from clinical samples.

BACKGROUND

Meropenem is an ultra-broad spectrum antibiotic. It is a beta-lactam and belongs to the subgroup of carbapenem, similar to imipenem and ertapenem.

Gentamicin is an aminoglycoside antibiotic which is synthesized by *Micromonospora*, a genus of Gram-positive bacteria widely present in the environment (water and soil). Gentamicin is one of the few heat-stable antibiotics that remain active even after autoclaving, which makes it particularly useful in the preparation of some microbiological growth media.

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

Mechanism of action

Meropenem is bactericidal except against Listeria monocytogenes where it is bacteriostatic. It inhibits its bacterial wall synthesis like other beta-lactam antibiotics. In contrast to other beta-lactams, it is highly resistant to degradation by beta-lactamases or cephalosporinases. Resistance generally arises due to mutations in penicillin binding proteins, production of metallo-beta-lactamases, or resistance to diffusion across the bacterial outer membrane. Unlike imipenem, it is stable to dehydropeptidase-1 and can therefore be given without cilastatin.

Gentamicin is a bactericidal antibiotic that works by binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis

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 VRE BROTH BASE AND VRE AGAR BASE

Selective media for the isolation of Vancomycin Resistant Enterococci (VRE) and High Level Aminoglycoside Resistant *Enterococci* (HLARE) from clinical samples. NB *Enterococci* containing the Van C genes will not be isolated on this medium.

The proliferation of *enterococci*, resistant to many commonly used antimicrobials is on the increase. The recent emergence of VRE is of great concern as enterococci can cause bacteraemia, endocarditis and urinary tract infections. The use of VRE Broth Base and VRE Agar Base complies with recommendations from the Centre for Disease Control and Prevention (CDC) to detect VRE infection in its early stages.

Resistant *enterococci* can be isolated either directly by

inoculation of the clinical sample onto supplemented VRE Agar, or indirectly isolated with a selective enrichment through VRE Broth followed by inoculation onto supplemented VRE Agar. VRE Agar Base contains an indicator system to detect the growth of aesculin-hydrolysing organisms. Enterococci produce black zones around the colonies from the formation of black iron phenolic compounds derived from aesculin-hydrolyis products and ferrous iron.

Toku-e has found three antibiotic supplements to selectively isolate antibiotic resistant populations amongst pathogenic *Enterococci*:

Meropenem is used at 2 mg/l in VRE Broth Base , and 1 mg/l in VRE Agar Base for the suppression of contaminating flora, particularly Gram-negatives and *Enterococcus gallinarum*. It has been reported that some *Enterococcus faecalis* can be sensitive to meropenem. To isolate these strains the level of meropenem may need to be reduced, or the supplement omitted from the formulation.

Gentamicin is used at 512 mg/l in VRE Agar Base for the selective isolation of HLARE.

Vancomycin is used at 6 mg/l in VRE Agar Base for the selective isolation of VRE.

Content concentrations

Typical Formula*	**mg/litre**
VRE Broth Base	
Calf brain infusion solids	12.5
Beef heart infusion solids	5
Proteose peptone	10
Glucose	2
Sodium chloride	5
Disodium phosphate	2.5
Final pH 7.4 ± 0.2 @ 25°C	
VRE Agar Base	
Tryptone	20
Yeast extract	5
Sodium chloride	5
Sodium citrate	1
Aesculin	1
Ferric ammonium citrate	0.5
Sodium azide	0.15
Agar	10
Final pH 7.0 ± 0.2 @ 25°C	
VRE Selective Supplement	
Meropenem	2†
Gentamicin	512†
Vancomycin	6†

* Adjusted as required to meet performance standards

† Adjusted as in the preparation

Table 1 - Typical Formula for VRE Broth Base, VRE Agar Base and supplements

METHOD

Preparation

VRE Broth

Suspend appreciate amount of VRE Broth Base in distilled water. Warm to dissolve completely, sterilise by autoclaving at 121°C for 15 minutes and cool to 50°C. Supplement the medium as shown in the table below. Then mix well and distribute into final sterile containers.

VRE Agar

Suspend appreciate amount of VRE Agar Base in distilled water. Warm to dissolve completely, sterilise by autoclaving at 121° C for 15 minutes and cool to 50°C. Supplement the medium as shown in the table below. Then Mix agar well and distribute into sterile Petri dishes.

Supplement	VRE Agar Base		VRE Broth
	VRE's	HLARE's	
Meropenem	1 mg/litre	-	2 mg/litre
Gentamicin	-	512 mg/litre	-
Vancomycin	6 mg/litre	-	-

Protocol

1. Prepare the medium from Columbia Blood Agar Base, Streptocccus Selective Supplement and Defibrinated Horse Blood, according to the preparation.

2. Inoculate the plates in the normal way and incubate at 35°C overnight in an atmosphere enriched with 5% carbon dioxide or anaerobically.*

3. Confirm that the colonies are *streptococci* by microscopy, biochemical or serological tests.

* Improved haemolytic reactions are achieved by anaerobic incubation. Gram-positive anaerobic cocci (*Peptostreptococcus* and *Peptococcus* species) would be selectively isolated under these conditions.

Quality control

VRE Broth:

Positive control:	Expected results
Enterococcus faecalis NCTC 12201	Growth
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

VRE Agar:

Positive control:	Expected results
Enterococcus faecalis NCTC 12201	Growth
Negative controls:	
Enterococcus faecalis ATCC® 33186	Inhibited
Escherichia coli ATCC® 25922*	Inhibited

HLARE Agar:

Positive Control:	Expected results
Enterococcus faecalis ATCC® 51299*	Growth
Negative control:	
Enterococcus faecalis ATCC® 29212*	Inhibited

REFERENCES

1. King, W. K. (1996) Bug Bytes Vol. 2 No. 19.

2. CDC Preventing the spread of vancomycin resistance: a report from the Hospital Infection Control Practices Advisory Committee (1994). Fed Regist. May 17.

3. Gold, H. S. & Moellering R.C. Jr. (1996) N. Engl. J. Med.; 335(19): 1445-53.

4. Weinbren, M. J., Johnson, A.P. & Woodford, N. (2000) J. Antimicrobial Chemotherapy; 45 :404-405.