ENTEROCOCCOSEL BROTH with 6 µg/mL VANCOMYCIN

- For in vitro use only - Catalogue No. TE60

Our Enterococcosel Broth with 6-µg/mL of vancomycin can be used as an enrichment broth for the detection of vancomycin resistant enterococci (VRE).

The detection of VRE is of growing importance due to the prevalence of enterococci as nosocomial pathogens. Although, VRE are relatively non-virulent the real danger lies in their potential to transfer resistance genes via plasmids or transposons to other organisms. To prevent such occurrences, detection, isolation, and treatment of infected individuals is of paramount importance.

Enterococcosel Agar, a modified version of Isenberg's bile esculin agar, was designed for the selective isolation of group D streptococci, and enterococci. In 1924, Rochaix noted the value of using esculin hydrolysis as a defining characteristic of enterococci. The resistance of *Enterococcus* species to bile and sodium azide was demonstrated on mediums devised by Meyer & Schonfeld and Isenberg et al., respectively. Enterococcosel Agar takes advantage of these characteristics making it a superior selective, differential isolation medium for enterococci. Enterococcosel broth has the same formulation as Enterococcosel agar with the agar omitted.

Enterococcosel Broth contains numerous peptones and extracts which provide the organism with nitrogen, amino acids, and other trace elements important for growth. Esculin is present in the medium to detect the bacterial enzyme, esculinase. Hydrolysis of esculin releases glucose and esculetin as end products; visual detection is possible since esculetin reacts with ferric ammonium citrate in the medium to form a brown-black phenolic iron complex which turns the broth brownish-black. The selectivity of the medium is due to the presence of bile (Oxgall) and sodium azide contained in the medium. Bile inhibits the majority of gram-positive organisms with the exception of group D streptococci and enterococci, while sodium azide inhibits the growth of gram-negative organisms. The 6-µg/mL of vancomycin present in the medium is the ideal concentration of antibiotic for screening VRE.

The addition of vancomycin to Enterococcosel Broth for the screening of VRE was described in 1995 by Van Horn et al. Their study showed that the medium gave rapid selective isolation and detection of VRE from surveillance specimens.

Formula per Litre of Medium

Pancreatic Digest of Casein	17.0 g
Papaic Digest of Animal Tissue	3.0 g
Yeast Extract	5.0 g
Sodium Chloride	5.0 g
Oxgall	10.0 g
Esculin	1.0 g
Ferric Ammonium Citrate	0.5 g
Sodium Azide	0.25 g
Vancomycin	6.0 mg

pH 7.1 ± 0.2

Recommended Procedure

1. Allow medium to adjust to room temperature prior to inoculation.
2. Using a direct inoculum from the specimen inoculate the broth. If the specimen is contained on a swab (rectal/perineal), the swab may be directly immersed in the medium. If the swab is used for inoculating more than one type of media, emulsify the swab in 1-mL of sterile saline. Mix well to obtain an even homogenous suspension and transfer 100-µL

of the suspension into the Enterococcosel broth.
3. Incubate aerobically at 35°C.
4. Examine broth after 24 hours. If the broth is turbid and/or changes to a brownish-blackish coloration subculture the broth onto an appropriate VRE screening agar (i.e. BHI with vancomycin) to obtain isolated colonies for further testing.
5. If no growth is observed re-incubate tubes an additional 24 hours before discarding.

Interpretation of Results

A positive presumptive result for VRE is turbidity in Enterococcosel Broth with 6-µg/mL of Vancomycin with esculin hydrolysis, which is observed as a darkening of the medium from an yellow-amber color to brownish-black or black color.

A negative result for VRE would be no turbidity or color change in the broth.

Additional biochemical and/or serological tests should be performed on isolated colonies from pure culture in order to complete identification.

- *Do not incubate beyond the recommended times or resistant non-VRE organisms may overcome inhibition and give erroneous results*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
Enterococcus faecalis ATCC 51299	Turbidity with blackening of medium
Enterococcus faecalis ATCC 29212	Complete inhibition with no color change

Storage and Shelf Life

Our Enterococcosel Broth with 6-µg/mL of Vancomycin should be stored away from direct light at 4 to 8°C. Under these conditions this medium has a shelf life of 8 weeks from the date of manufacture.

References

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5. Van Horn KG, Gedris CA, Rodney KM. Selective isolation of vancomycin-resistant enterococci. J Clin Micro 1996; 34:924-7.
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