



ENTEROCOCCOSEL AGAR w 6 µg/mL VANCOMYCIN

- For in vitro use only -

Catalogue No. PE56

Our Enterococcosel Agar with 6-µg/mL of vancomycin can be used as a primary screening plate 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, is 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 Agar 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 that appears as a brown halo around colonies. 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. Non-VRE and group D streptococci are completely inhibited on Enterococcosel Agar with 6 µg/mL of Vancomycin

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
Agar	13.5 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 streak as to obtain isolated colonies, or alternatively if the specimen is contained on a swab (rectal/perineal), directly streak the surface of the medium using the swab.
3. Incubate aerobically at 35°C.
4. Examine plates after 24 hours.
5. If no growth is observed re-incubate plates an additional 24 hours before discarding.

Interpretation of Results

A positive presumptive result for VRE is growth on Enterococcosel Agar with 6-µg/mL of Vancomycin with esculin hydrolysis, which is observed as a darkening of the medium. VRE colonies are small and translucent with brownish-black halos. A negative result for VRE would be no growth on Enterococcosel Agar.

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

- *Ensure that positive and negative controls are run in conjunction with specimen samples to ensure the efficacy of the media due to the possibility of vancomycin deterioration over time*
- *Do not incubate beyond the recommended times or 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. A suspension equivalent to a 0.5 McFarland standard is prepared for each organism and a 10-µL spot inoculum is placed onto individual plates.

Organism	Expected Result
<i>Enterococcus faecalis</i> ATCC 51299	Growth with blackening of medium
<i>Enterococcus faecalis</i> ATCC 29212	Complete inhibition

Storage and Shelf Life

Our Enterococcosel Agar with 6-µg/mL of Vancomycin should be stored away from direct light at 4°C to 8°C. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 6 weeks from the date of manufacture.

References

1. Rochaix. R Soc Biol 1924; 90:771.
2. Meyer, Schonfeld. Zentralbl Bakt Parasit Infect Hyg Abt Orig 1926; 99:402.
3. Isenberg H, Goldberg D, Sampson J. Laboratory studies with a selective enterococcus medium. Appl Micro 1970; 20:433.
4. Jensen BJ. Screening specimens for vancomycin-resistant *Enterococcus*. Lab Med 1996; 27:53-5.
5. CEQA-AGAR. Guidelines for the testing and reporting of antimicrobial susceptibilities of vancomycin resistant enterococci. Health Protection Branch – Laboratory Centre for Disease Control, 1998.
6. NCCLS. M7-A5 Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 5th ed. Pennsylvania: NCCLS, 2000.
7. MacFaddin, JF. Biochemical tests for the identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

Original: April 2001

Revised / Reviewed: October 2014