m-ENTEROCOCCUS AGAR
w 6 µg/mL VANCOMYCIN

- For in vitro use only - Catalogue No. PE58

Our m-Enterococcus Agar with 6 µg/mL Vancomycin is a selective medium for the screening and isolation of Vancomycin-Resistant Enterococci (VRE).

m-Enterococcus Agar was initially developed by Slanetz, Bent, and Bartley for the enumeration of enterococci, the addition of triphenyltetrazolium chloride (TTC) was described later by Slanetz and Bartley. TTC is a colorless dye used as an indicator of bacterial growth. Organisms capable of growing on the medium reduce TTC to produce the insoluble, red-colored compound formazan thereby producing pink or red colonies. A variety of peptones, and extracts provide the necessary growth factors to elicit sustained growth of the desired microorganism. The selective agent, sodium azide, inhibits growth of gram-negative organisms while the addition of 6 µg/mL of vancomycin makes it a suitable screening medium for VRE. Non-VRE and group D streptococci are completely inhibited on this medium.

VRE testing using m-Enterococcus medium with 6 µg/mL of vancomycin was described by Van Horn, Gedris, and Rodney in 1996. The agar formulation was part of the guidelines for VRE testing recommended by the Canadian External Quality Assessment – Advisory Group on Antibiotic Resistance (CEQA-AGAR) in 1998.

Formula per Litre of Medium

Yeast Extract	5.0 g
Casein Peptone	15.0 g
Dextrose	2.0 g
Soy Peptone	5.0 g
Potassium Phosphate	4.0 g
Sodium Azide	0.4 g
Agar	10.0 g
Triphenyltetrazolium Chloride	0.1 g
Vancomycin	6.0 mg

pH 7.2 ± 0.2

Recommended Procedure

1. Allow medium to warm to room temperature prior to inoculation.
2. Using an inoculum from the specimen, streak the plate as to obtain isolated colonies. If the specimen is contained on a swab, roll the swab several times over a small area near the edge of the plate. Then using a sterile loop, proceed to streak the plate starting where the swab was inoculated.
3. Incubate aerobically at 35°C.
4. Examine after 24 hours.
5. If negative, re-incubate plates further for an additional 24 hours before discarding.

Interpretation of Results

Vancomycin-resistant enterococci produce small, pink or red colonies on m-Enterococcus Agar. Growth of most other organisms is inhibited although some organisms capable of growing on the medium will also appear pink and further testing is needed to differentiate these organisms from VRE.

Potential VRE isolates should be subcultured onto a non-selective blood plate so that further testing can be performed on colonies from pure culture. Vancomycin resistance should be confirmed on BHI Agar with 6 µg/mL of Vancomycin (Dalynn PB59) as prescribed by the NCCLS M7 document. A MIC determination should also be made since some enterococci show a intrinsic resistance to vancomycin and these organisms are of less interest since their resistance

genes cannot be transferred.

Additional biochemical and/or serological tests should be performed on isolated colonies from pure culture in order to identify the organism to the species level.

- *Some enterococci such as E. gallinarum, E. casseliflavus and E. flavescens possess a low or intermediate intrinsic resistance to vancomycin and may grow on this medium. These organisms must be differentiated from VRE that demonstrate a high-level of acquired resistance to vancomycin*

Quality Control

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

Organism	**Expected Result**
Enterococcus faecalis ATCC 51299	Growth, pink to dark red colonies
Enterococcus faecalis ATCC 29212	Inhibition

Storage and Shelf Life

Our m-Enterococcus Agar with 6 µg/mL Vancomycin should be stored away from direct light at 4°C to 8°C with the medium side up to prevent excessive accumulation of moisture on the agar surface. Under these conditions, this medium has a shelf life of 10 weeks from the date of manufacture.

References

1. Slanetz LW, Bent DF, and Bartley CH. Use of the membrane filter technique to enumerate enterococci. Public Health Rep 1955; 70:67.
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3. Grey JW, Pedler SJ. J Hosp Infect 1992;21:1-14.
4. Van Horn KG, Gedris CA, Rodney KM. Selective isolation of vancomycin-resistant enterococci. J Clin Micro 1996;34:924-927.
5. Ofner-Agostini ME, Conly J, Paton S et al. Vancomycin-resistant enterococci (VRE) in Canada – results of the Canadian nosocomial infection surveillance program 1996 VRE point prevalence surveillance project, LCDC report. Can J Infect Dis 1997; 8:73-78.
6. CEQA-AGAR. Guidelines for the testing and reporting of antimicrobial susceptibilities of vancomycin resistant enterococci. Health Canada, Laboratory for Disease Control, 1998.
7. NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th Ed. NCCLS document M7-A5. Villanova, Pennsylvania, 2001.

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