



BHI AGAR with 6 µg/mL VANCOMYCIN

- For in vitro use only -

Catalogue No. PB59

Our BHI Agar with 6µg/mL of Vancomycin is used as a screening medium for Vancomycin-Resistant Enterococci (VRE).

The increasing prevalence of VRE as a nosocomial pathogen makes their detection of vital importance. Patients colonized with VRE are difficult to treat and can spread the organism quite quickly in an institutional setting. The two most medically important *Enterococcus* species are *E. faecalis* and *E. faecium*, although numerous other species show varying degrees of resistance to vancomycin.

The NCCLS has recommended that the simplest and most sensitive test for screening for VRE is the use of BHI Agar with 6 µg/mL of vancomycin. A spot inoculation from pure culture is prescribed along with follow up confirmatory tests.

Infusion from brain heart, peptones and dextrose provide essential growth factors; potassium phosphate helps to buffer the medium; while sodium chloride provides an isotonic growth environment for bacterial cells. The inclusion of 6-µg/mL of vancomycin screens for vancomycin resistant organisms including VRE. Other possible isolates include *Lactobacillus* species, *Enterococcus casseliflavus*, and *Enterococcus gallinarum* that possess an intrinsic resistance to vancomycin. The detection and isolation of vancomycin-intermediate *Staphylococcus aureus* (VISA) may also be possible using this medium.

Formula per Litre of Medium

Brain Heart, Infusion from (Solids)	8.0 g
Peptones	21.0 g
Sodium Chloride	5.0 g
Dextrose	2.0 g
Sodium Phosphate.....	2.5 g
Agar.....	13.5 g
Vancomycin	6.0 mg

pH 7.4 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature prior to inoculation.
2. Prepare a pure organism suspension in TSB equivalent to that of a 0.5 McFarland Standard.
3. Using a 10-µL loop, spot inoculate the surface of the BHI Agar with Vancomycin plate.
4. Incubate aerobically at 35°C.
5. Examine after a full 24-hour incubation period.

Interpretation of Results

Any growth at the spot of inoculation is considered a presumptive positive for VRE and requires that confirmatory testing be done on the isolates. These tests include a gram stain, biochemical tests for species-level identification, and determination of the vancomycin MIC.

No growth at the spot of inoculation is a negative result for VRE.

- *BHI Agar with Vancomycin is not entirely specific for van-A and van-B type resistance. Other organisms with intrinsic (vanC), intermediate resistance to vancomycin may demonstrate weak growth on this medium*
- *Vancomycin-intermediate Staphylococcus aureus (VISA) also grow on this medium and may represent the simplest and least expensive method for its detection*
- *A full 24 hour incubation is necessary to detect all resistant isolates; incubating and interpreting plates after the 24-hour period is not recommended*

Quality Control

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

<u>Organism</u>	<u>Expected Result</u>
<i>Enterococcus faecalis</i> (VRE) ATCC 51299	Growth
<i>Enterococcus faecalis</i> ATCC 29212	Inhibition

Storage and Shelf Life

Our BHI Agar with 6 µg/mL 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 5 weeks from the date of manufacture.

References

1. Swenson JM, et al. Development of a standardized screening method for detection of vancomycin-resistant enterococci. *J Clin Microbiol* 1994; 32:1700-4.
2. Tenover FC, et al. Characterization of staphylococci with reduced susceptibilities to vancomycin and other glycopeptides. *J Clin Microbiol* 1998; 36:1020-7.
3. Smith TL, et al. Emergence of vancomycin resistance in *Staphylococcus aureus*. *NEJM* 1999; 340:517-23.
4. NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5. Wayne, PA: NCCLS, 2000.
5. NCCLS. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A7. Wayne, PA: NCCLS, 2000.
6. NCCLS. Performance standards for antimicrobial susceptibility testing. Tenth informational supplement M100-S10. Wayne, PA: NCCLS, 2000.

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