

Brain Heart Infusion Agar with Vancomycin Screen Test for VISA and VRSA

Preanalytical Considerations

I. PRINCIPLE

A screen test with BHI agar containing 6 µg/ml vancomycin (BHI-V6) can be used to detect vancomycin-intermediate *Staphylococcus aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) isolated from clinical or surveillance cultures. VISA have vancomycin MICs of 8-16 µg/ml whereas VRSA have vancomycin MICs 32 µg/ml or greater.

An aliquot of an inoculum suspension standardized to match a McFarland 0.5 is inoculated onto a section of a BHI-V6 plate. Following 24 hours incubation, growth on the agar indicates that the *S. aureus* may be a VISA or VRSA. MIC testing for vancomycin using an acceptable non-automated method is then performed to determine if the organism growing on BHI-V6 is VISA or VRSA. If results remain consistent for VISA or VRSA, the isolate is sent to a local public health laboratory and/or to the Centers for Disease Control and Prevention (CDC) for further study. When a VISA or VRSA is confirmed or highly suspected, results are communicated to the patient's physician and infection control as well as to appropriate public health authorities.

II. SPECIMEN

Prepare inoculum from four or five isolated colonies of similar colony morphology grown overnight (18-24h) on non-selective agar medium (e.g., BAP, chocolate)

III. MATERIALS

A. Media and Reagents

1. BHI agar plates with 6 µg/ml vancomycin
Store at 2-8°C
2. Mueller-Hinton broth or 0.85% NaCl (3.0 - 5.0 ml aliquots)
Store at 2-8°C

B. Supplies

1. Sterile cotton tipped swabs
2. Sterile plastic pipettes
3. Micropipette tips (if micropipette used)
4. McFarland 0.5 turbidity standard

C. Equipment

1. 10 µl (0.01 ml) micropipette
2. Vortex mixer

3. 35°C ambient air incubator

Analytical Considerations

IV. QUALITY CONTROL

- A. Quality control strains
 1. Vancomycin susceptible control
Enterococcus faecalis ATCC 29212
 2. Vancomycin resistant control
Enterococcus faecalis ATCC 51299
- B. Frequency of testing QC strains
 1. Weekly
If the vancomycin screen test is performed at least once a week and 20 to 30 days of daily QC testing has been performed with *E. faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 and results are acceptable as defined in NCCLS M7-A6.
 2. Daily
If vancomycin screen test is performed less frequently than once a week
- C. Record all results on quality control worksheet

V. Procedure

- A. Inoculum preparation
Using a loop or swab, transfer colonies to broth or saline to obtain an organism suspension that matches a McFarland 0.5 turbidity standard (1.5×10^8 CFU/ml); vortex thoroughly.
- B. Inoculation and incubation
 1. Using a micropipette, spot a 10 μ l drop onto agar surface 10-15 mm in diameter. Alternatively, using a swab dipped in the suspension and expressed, spot a similar area or streak a portion of the plate. Test up to eight isolates/plate.
 2. Allow the inoculum to be absorbed into the agar.
 3. Invert plates and incubate at 35°C in an ambient air incubator
 4. Examine after overnight incubation. For isolates that show no growth, reincubate until a full 24 h of incubation has occurred and examine again.
- C. Reading plates
Examine carefully with transmitted light for >1 colony or light film of growth.
- D. Confirmatory testing if BHI-V6 demonstrates >1 colony:
 1. Check purity of the culture

2. Confirm identification of the isolate
3. Retest the isolate using a non-automated MIC method. Acceptable methods include
 - a. Reference broth microdilution
 - b. Agar dilution
 - c. Agar gradient diffusion (Etest; use a 0.5 McFarland inoculum and Mueller-Hinton agar)
4. Send *S. aureus* with vancomycin MICs 4 µg/ml or greater to a public health laboratory and/or CDC where results will be confirmed using several methods. **SAVE isolate.**

Postanalytical Considerations

VI. REPORTING

- A. Interpretation
 1. No growth - vancomycin susceptible
 2. Growth of > 1 colony – presumptive VISA or VRSA
- B. Reporting
 1. Report presumptive results
Example:
Staphylococcus aureus, presumptive VISA (or VRSA) based on screen test; confirmatory tests pending.
 2. Following confirmation using a non-automated MIC method for vancomycin, report supplemental results
Example:
Staphylococcus aureus, VISA (or VRSA); isolate sent to public health department and CDC for additional studies.
 3. NOTIFY patient's physician, infection control, local public health department and CDC (SEARCH@CDC.gov) of *S. aureus* with vancomycin MIC of 4 µg/ml or greater

VII. PROCEDURE NOTES

- A. Up to eight isolates can be tested per BHI-V6 plate (100 mm diameter) if extreme care is taken to prevent overlapping of inocula.
- B. The medium described here is to be used for testing *S. aureus* that have been isolated in culture and not as a primary plating medium for surveillance or other specimens.

VIII. LIMITATIONS

- A. The vancomycin screen plate does not determine the level of vancomycin resistance or the vancomycin phenotype.
- B. Vancomycin-resistant organisms other than VISA or VRSA (e.g. gram-negative bacteria, VRE, *Leuconostoc* spp., *Lactobacillus* spp., *Pediococcus* spp., *Erysipelothix rhusiopathiae*, yeasts) may grow on BHI-V6. It is critical to confirm identity and purity of any isolate that grows on BHI-V6.

REFERENCES

1. CDC. 2004. Brief Report: Vancomycin-resistant *Staphylococcus aureus*---New York, 2004. Morb Mortal Wkly Rep. 53:322-323.
2. NCCLS. 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 6th ed. Approved Standard M7-A6. NCCLS, Wayne, PA.
3. CLSI/NCCLS. 2005. Performance standards for antimicrobial susceptibility testing. Fifteenth informational supplement. M100-S15. CLSI/NCCLS, Wayne, PA.
4. Tenover, F. C., M. V. Lancaster, B. C. Hill, C. D. Steward, S. A. Stocker, G. A. Hancock, C. M. O'Hara, S. K. McAllister, N. C. Clark, and K. Hiramatsu 1998. Characterization of staphylococci with reduced susceptibilities to vancomycin and other glycopeptides J Clin Microbiol. 36:1020-7.

CDC Websites:

VISA/VRSA - Vancomycin-Intermediate/Resistant *Staphylococcus aureus*
Laboratory Testing Algorithm http://www.cdc.gov/ncidod/hip/Lab/FactSheet/visa_vrsa_algo.htm

VISA/VRSA - Vancomycin-Intermediate/Resistant *Staphylococcus aureus*
Laboratory Detection Fact Sheet <http://www.cdc.gov/ncidod/hip/Lab/FactSheet/vrsa.htm>