

QUALITY CONTROL PROCEDURES

I INTRODUCTION

Vancomycin Screen Agar is used to test enterococci for resistance to vancomycin and to predict the synergistic activity of this antimicrobial with an aminoglycoside antimicrobial.

II PERFORMANCE TEST PROCEDURE

1. Create a 0.5 McFarland suspension of 18 – 24 h enterococcal isolates in a tube of **Trypticase™** Soy Broth.
2. Inoculate representative samples with the cultures listed below.
 - a. Spot inoculate 10 µL (0.01 mL), allow to absorb into agar bed.
 - b. Incubate plates at 36 ± 1°C in an aerobic atmosphere.
 - c. Include plates of a previously tested lot of TSA with 5% Sheep Blood as controls for all strains.
3. Examine plates after 24 h for growth and selectivity.
4. Expected Results

Organisms	ATCC™	Recovery
* <i>Enterococcus faecalis</i>	29212	–
* <i>Enterococcus faecalis</i>	51299	+

*Recommended organism strain for User Quality Control.

– = No growth or one colony

+ = Growth greater than one colony

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under “Product Deterioration.”
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.4 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Vancomycin Screen Agar is used to test enterococci for resistance to vancomycin and to predict the synergistic activity of this antimicrobial with an aminoglycoside antimicrobial.

V SUMMARY AND EXPLANATION

Enterococci are known to cause a wide variety of infections. Most commonly they infect the urinary tract, abdomen, bloodstream, endocardium, biliary tract, burn wounds and in-dwelling catheters.¹ *Enterococcus faecalis* causes 80 to 90% of infections, while *E. faecium* causes the majority of the remainder.² Today the enterococci are the fourth leading cause of hospital acquired infection and the third leading cause of bacteremia in the United States.³ The case/fatality rates for enterococcal bacteremia range from 12 to 68% with death due to sepsis in 4 to 50% of the cases.⁴

Treatment of enterococcal infections with either penicillin or vancomycin alone fails to kill enterococci resulting in relapse of infection.⁵ Enterococci for years were known to have low intrinsic resistance to a variety of β-lactam as well as aminoglycoside antibiotics.⁶ The addition of an aminoglycoside to which the isolate has demonstrated susceptibility results in both *in vitro* and *in vivo* synergism producing a bactericidal effect.⁷ This synergistic effect is thought to be due to the penicillin or vancomycin damaging the integrity of the cell wall, thus allowing the aminoglycoside to penetrate and inhibit bacterial protein synthesis.⁸ The emergence of resistance to vancomycin (≥ 6 µg/mL)⁹ may result in the failure of vancomycin-aminoglycoside combinations to eradicate the infecting organisms. Therefore, testing for resistance to vancomycin is important. The use of a Brain Heart Infusion Agar (BHIA) containing vancomycin (6 µg/mL) is recommended by the Clinical and Laboratory Standards Institute (CLSI) for testing resistance.¹⁰

VI PRINCIPLES OF THE PROCEDURE

The Brain Heart Infusion Agar base is a general-purpose medium suitable for the cultivation of a wide variety of microorganisms.

The meat infusion solids and peptones are sources of organic nitrogen, carbon, sulfur, vitamins, and trace substances. Dextrose is the carbohydrate source. The medium is buffered through the use of disodium phosphate. Vancomycin at 6 µg/mL is used to detect resistance to vancomycin.¹⁰ The Food, Drug & Cosmetic (FD&C) dye is inert and added for easy visual identification of this vancomycin-containing medium.

VII REAGENTS

Vancomycin Screen Agar

Approximate Formula* Per Liter Purified Water

Brain Heart, Infusion from (solids)	8.0 g	Disodium Phosphate	2.5 g
Peptic Digest of Animal Tissue.....	5.0 g	Agar	13.5 g
Pancreatic Digest of Casein.....	16.0 g	FD&C Yellow #5 Dye	0.56 g
Sodium Chloride	5.0 g	Vancomycin.....	6.0 mg
Dextrose	2.0 g		

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding. If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Storage Instructions: On receipt, store plates in the dark at 2 – 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 – 8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

This medium is not intended for use with specimens or mixed cultures. The organism to be tested must first be in pure culture and presumptively identified as *Enterococcus* species.

IX PROCEDURE

Material Provided: Vancomycin Screen Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

1. Prepare the inoculum by suspending several well-isolated colonies of the enterococcal isolate from an 18 to 24 h plate culture into a tube of **Trypticase™** Soy Broth and adjust the turbidity to be equivalent to a 0.5 McFarland turbidity standard.
2. Spot inoculate the plate with 10 µL of the adjusted suspension.¹¹
3. Allow the inoculum spot to absorb into the agar surface.
4. Inoculate a growth control plate, such as a **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II), in the same manner.
5. Incubate plates at 35 ± 2°C aerobically for a full 24 h.

User Quality Control: See “Quality Control Procedures.”

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory’s standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Following a full 24 h of incubation, observe plates for growth.

Growth Control plate: Growth indicates viable test organisms in the inoculum broth suspension and the test is valid. If there is no growth, the test is invalid and must be repeated.

Vancomycin Screen Agar: Growth (> one colony) indicates that the antimicrobial agent may not be synergistic in combination therapy. No growth (≤ one colony) indicates that synergy may be predicted.¹⁰

XI LIMITATIONS OF THE PROCEDURE

This product is intended for use with *Enterococcus* species. Occasionally enterococcal isolates with borderline antimicrobial susceptible MICs may show growth. This product provides a screening method for predicting synergistic contributions of vancomycin with aminoglycosides. It is recommended that synergy studies be conducted with any enterococcal isolates that grow on the screen agar prior to commencing or ruling out combination therapy with vancomycin. The checkerboard technique and time-kill tests are definitive methods for determining synergy.¹¹

To determine the precise concentration of vancomycin to which an enterococcal isolate is resistant, minimal inhibitory concentration testing should be performed as recommended in CLSI document M7-A8.¹⁰ The determination of the phenotypic type of resistance to vancomycin (VanA, VanB or VanC) is recommended in order to optimize infection control measures.^{12,13}

The enterococcal test strain may be resistant to penicillin and ampicillin from the alteration of penicillin-binding proteins or the production of β-lactamase. Consult the CLSI document for appropriate test methods.¹⁰ The synergistic activity of a penicillin and aminoglycoside combination cannot be predicted from the vancomycin results.

XII PERFORMANCE CHARACTERISTICS

The agar screen test procedure for detecting vancomycin resistant enterococci recommended by CLSI was performed in-house with 50 *Enterococcus* isolates using **BBL™** screen medium that contained BHIA with 6 µg/mL vancomycin. The 50 enterococcal isolates consisted of 21 *E. faecalis*, 19 *E. faecium*, 4 *E. gallinarum*, 2 *E. raffinosus*, 1 *E. casseliflavus*, 1 *E. mundtii*, and 2 *E. avium*. Of these 50 isolates 28 (56%) were resistant to vancomycin. Phenotypic characterization included the use of agar and/or broth dilution to establish vancomycin MICs. There was 100% correlation between test results and expected results.


Reproducibility studies (3x/day for 3 days) were done at two field sites with 15 enterococcal isolates. The 15 isolates consisted of 7 *E. faecalis*, 4 *E. faecium*, 1 *E. gallinarum*, 1 *E. avium*, 1 *E. casseliflavus*, and 1 *E. raffinosus*. Of these 8 (53%) were resistant to vancomycin. Phenotypic characterization included the use of agar and/or broth dilution to establish vancomycin MICs. Here there was also 100% correlation between the test results and the expected results.


XIII AVAILABILITY

Cat. No.	Description
222204	BBL™ Vancomycin Screen Agar, Pkg. of 10 plates

XIV REFERENCES

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