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
<table>
<thead>
<tr>
<th>Organisms</th>
<th>ATCC®</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>29212</td>
<td>–</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>51299</td>
<td>+</td>
</tr>
</tbody>
</table>
   *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. E. faecalis causes 80 to 90% of infections, while E. faecium causes the majority of the remainder. Today the enterococci are the fourth leading cause of hospital acquired infection and the third leading cause of bacteremia in the United States. The case/fatality rates for enterococcal bacteremia range from 12 to 68% with death due to sepsis in 4 to 50% of the cases.

Treatment of enterococcal infections with either penicillin or vancomycin alone fails to kill enterococci resulting in relapse of infection. Enterococci for years were known to have low intrinsic resistance to a variety of β-lactam as well as aminoglycoside antibiotics. 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. 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. The emergence of resistance to vancomycin (≥ 6 µg/mL) 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.

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. 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

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain Heart, infusion from (solids)</td>
<td>8.0 g</td>
</tr>
<tr>
<td>Peptic Digest of Animal Tissue</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Pancreatic Digest of Casein</td>
<td>16.0 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Disodium Phosphate</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Agar</td>
<td>13.5 g</td>
</tr>
<tr>
<td>FD&amp;C Yellow #5 Dye</td>
<td>0.56 g</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>6.0 mg</td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>222204</td>
<td>BD BBL™ Vancomycin Screen Agar</td>
</tr>
</tbody>
</table>
XIV REFERENCES


Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/id.