INTENDED USE
Remel Bile Esculin Azide Agar w/ 6 µg/ml Vancomycin is a solid medium recommended for use in qualitative procedures as a screening method for primary isolation and presumptive identification of vancomycin-resistant enterococci (VRE) from surveillance cultures.

SUMMARY AND EXPLANATION
Members of the genus Enterococcus are normal residents of the gastrointestinal and biliary tracts. Enterococci have become significant agents of human disease, largely because of their evolving resistance to antimicrobial agents. Risk factors related to nosocomial infection include previous therapy with vancomycin or multiple antimicrobial agents, severe underlying disease or immunosuppression, and intra-abdominal surgery. Reports of outbreaks and endemic infections demonstrate that VRE transmission can occur via patient-to-patient contact, healthcare workers, contaminated environmental surfaces, or patient-care equipment. VRE surveillance-screening has been found to facilitate earlier identification of colonized patients leading to more efficient containment of the organism. Timely identification of VRE is improved with the use of primary isolation medium for surveillance cultures. Sahm et al. demonstrated that Bile Esculin Azide Agar w/ 6 µg/ml Vancomycin provides for rapid presumptive identification of VRE. Esculin in the medium allows for early recognition of presumptive enterococcal isolates, selective agents inhibit growth of most commensal microbial flora, and vancomycin provides additional selectivity against vancomycin-sensitive enterococci and other gram-positive organisms.

PRINCIPLE
Group D streptococci and enterococci hydrolyze esculin in the presence of bile, resulting in the production of esculetin and dextrose. Ferric ammonium citrate supplies ferric ions which react with esculetin to form a black-brown complex. Sodium azide and 1% oxgall (equivalent to 10% bile) are selective agents inhibitory to gram-negative bacilli and most gram-positive bacteria other than group D streptococci. Vancomycin (6 µg/ml) is added to select for resistant strains of enterococci.

REAGENTS (CLASSICAL FORMULA)*
Casein Peptone ........................................................... 17.0 g
Oxgall (10% Bile) ........................................................ 10.0 g
Yeast Extract ................................................................. 5.0 g
Sodium Chloride ............................................................ 5.0 g
Meat Peptone ................................................................ 3.0 g
Sodium Citrate .............................................................. 1.0 g

Esculin ............................................................................. 1.0 g
Ferric Ammonium Citrate ............................................0.5 g
Sodium Azide ............................................................0.25 g
Vancomycin ....................................................................6.0 mg
Agar . .........................................................................15.0 g
Demineralized Water .............................................1000.0 ml

pH 7.1 ± 0.2 @ 25°C
*Adjusted as required to meet performance standards.

PROCEDURE
1. Inoculate and streak the specimen as soon as possible (within 2 hours) after it is received in the laboratory.
2. Incubate plate aerobically or in 5-10% CO2 at 33-37°C for 24-48 hours.
3. Examine daily for the presence of colonies which produce a brown to black pigment diffusing into the medium.
4. Verify by Gram stain that esculin-positive colony is gram-positive cocci morphologically characteristic of streptococci or enterococci.
5. Subculture isolate to a nonselective medium, such as blood agar, for additional testing. Definitive identification of group D streptococci and enterococci requires additional biochemical and/or serological testing following established laboratory procedures. Consult appropriate references for further instructions.
6. Confirm vancomycin resistance using standardized susceptibility methods following established laboratory procedures. Consult appropriate references for further instructions.

INTERPRETATION OF THE TEST
Positive Test - Dark brown to black color around colonies and diffusing into the medium
Negative Test - No blackening of the medium

QUALITY CONTROL
All lot numbers of Bile Esculin Azide Agar w/ 6 µg/ml Vancomycin have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL
Enterococcus faecalis ATCC®51299
Enterococcus faecalis ATCC®29212
Escherichia coli ATCC®25922

INCUBATION
Aerobic, 24 h @ 33-37°C
Aerobic, 24 h @ 33-37°C
Aerobic, 24 h @ 33-37°C

RESULTS
Growth, blackening of medium
No growth
No growth

(Continued on back)
LIMITATIONS
1. This medium is recommended as a screening method for primary isolation of bile esculin-positive, vancomycin-resistant, gram-positive cocci and is not intended for use as a method of antimicrobial susceptibility testing.
2. Esculin-positive organisms other than enterococci (e.g., *Pediococcus*, *Leuconostoc*, and *Lactobacillus*) may grow on this medium. Further biochemical and serological testing is required for definitive identification. Consult appropriate references for further instructions.1,2
3. Some organisms may overcome the inhibitory effects of this medium on initial isolation. Confirm vancomycin resistance using an approved method.7
4. The absence of suspect colonies does not rule out the presence of VRE. *Enterococcus casseliflavus* and *Enterococcus gallinarum* are intrinsically resistant to vancomycin due to the presence of the *vanC* gene which may not be expressed when testing on this medium.

PERFORMANCE CHARACTERISTICS6
Bile Esculin Azide Agar w/ 6 µg/ml Vancomycin (BEAV) was evaluated with a total of 2,777 specimens from 2,127 inpatients (2,264 urine and 513 stools, for *C. difficile* analysis). No yeast, gram-negative bacteria, or vancomycin-susceptible gram-positive bacteria grew on the medium. Of 378 specimens that yielded black colonies, 147 were gram-positive bacilli as determined by Gram stain of BEAV growth. Of the remaining 231 gram-positive cocci isolated, 25 were identified as probable *Pediococcus* spp. (PYR −, LAP +) and 206 were VRE (PYR +, LAP +). VRE were speciated, susceptibility tested, and all profiles were confirmed by PCR analysis for *vanA*, *vanB*, *vanC1*, and *vanC2* genes. Of 121 individual patient isolates examined in detail, 83 *E. faecium*, 33 *E. gallinarum*, and 5 *E. casseliflavus* were included. Vancomycin MIC values for these isolates ranged from 8 to >256 µg/ml.

VRE stock isolates from a variety of geographical locations, which had been previously identified and characterized according to *van* gene content, were also evaluated in this study. All 47 strains exhibited growth on this medium.

<table>
<thead>
<tr>
<th>Species Identification</th>
<th>Gene Content</th>
<th>No. of Strains</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterococcus faecium</em></td>
<td>vanA</td>
<td>4</td>
<td>512</td>
</tr>
<tr>
<td></td>
<td>vanB</td>
<td>5</td>
<td>≥4</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>vanA</td>
<td>1</td>
<td>512</td>
</tr>
<tr>
<td></td>
<td>vanB</td>
<td>10</td>
<td>≥4</td>
</tr>
<tr>
<td><em>Enterococcus gallinarum</em></td>
<td>vanC1</td>
<td>11</td>
<td>4-16</td>
</tr>
<tr>
<td><em>Enterococcus casseliflavus</em></td>
<td>vanC2</td>
<td>10</td>
<td>4-8</td>
</tr>
</tbody>
</table>

BIBLIOGRAPHY

Refer to the front of Remel *Technical Manual of Microbiological Media* for General Information regarding precautions, product storage and deterioration, specimen collection, transportation, materials required, quality control, and limitations.

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IFU 1186, Revised February 5, 2008

Printed in U.S.A.