Comparison of Dalbavancin MIC Values Determined by Etest (AB BIODISK) and Reference Dilution Methods Using Gram-Positive Organisms

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While standardized microdilution testing methodologies and quality control ranges exist for the novel glycolipopeptide dalbavancin, no testing methods have been described that are immediately available for routine use in clinical laboratories. In this study, we found that the dalbavancin Etest (AB BIODISK, Solna, Sweden) procedure demonstrated a high degree of agreement (100% within ±2 log₂ dilution steps) with the standardized broth microdilution method, validating the use of the Etest as an alternative test for investigational or clinical purposes following regulatory approval.

The continued emergence of resistance phenotypes among gram-positive pathogens has compromised the empirical usage of many targeted, traditional antimicrobials and necessitated the development of new agents displaying stability to the most commonly occurring resistance mechanisms. Among agents currently undergoing regulatory review is dalbavancin (formerly BI-397), a once-weekly, parenterally administered semi-synthetic glycopeptide derivative of the natural glycopeptide A-40,926 produced by a 3,3-dimethylaminopropyl amide substitution on the peptide carboxyl group (9). The mechanism of bactericidal activity is similar to that of other glycopeptides, via interference with bacterial cell wall biosynthesis through binding to the terminal α-alanyl-β-alanine of nascent peptidoglycan chains. Dalbavancin has demonstrated potent activity against clinically relevant aerobic and anaerobic gram-positive organisms, including oxacillin (methicillin)-resistant staphylococci, penicillin-resistant Streptococcus pneumoniae, and certain vancomycin-resistant enterococci (vanB phenotypes), and has proven efficacy in clinical trials with skin and soft-tissue infections (3, 4, 8, 14, 15).

The Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards [NCCLS]) reference broth microdilution MIC testing methodology and quality control (QC) guidelines for dalbavancin have been reported on previously (1, 2, 5, 6, 10). Importantly, the incorporation of the surfactant polysorbate-80 into dalbavancin-containing wells in reference frozen-form panels has been found to be critical for accuracy and reproducibility of MIC results (2, 5, 7).

The purpose of this investigation was primarily to validate the use of the Etest (AB BIODISK, Solna, Sweden) as a commercial antimicrobial susceptibility test method for determining the susceptibility of indicated bacterial species to dalbavancin, comparing results with those MICs generated by the broth microdilution method, and secondarily to investigate comparability with the agar dilution method (10, 12). Given the familiarity with the Etest among laboratories and the ease of use (and the current absence of dalbavancin disk diffusion or automated system methods), this validation would permit clinical laboratories to test dalbavancin in support of clinical trials, surveillance investigations, and patient therapy without having to rely on the use of the standardized, reference broth-based MIC method.

The study organism collection consisted of 200 gram-positive isolates including Staphylococcus aureus (90 total, including 40 resistant to oxacillin [methicillin] and 5 displaying reduced susceptibility to vancomycin [MICs, 4 to 8 µg/ml]), coagulase-negative staphylococci (CoNS) (20 total; 10 oxacillin [methicillin] resistant), Enterococcus spp. (20 total; 5 Enterococcus faecalis, 9 Enterococcus faecium, 6 others; 14 vancomycin resistant), beta-hemolytic streptococci (n = 37), Streptococcus pneumoniae (n = 20), and viridans group streptococci (n = 13).

Quality control strains (S. aureus ATCC 29213, S. pneumoniae ATCC 49619, and Enterococcus faecalis ATCC 29212) were included in each day of testing (five replicates each). Test methodologies included broth microdilution, agar dilution, and appropriate QC studies, performed according the technical details described in CLSI documents M7-A6, M23-A2, and M100-S16 (2, 11, 12). Broth microdilution testing of dalbavancin included the incorporation of 0.002% (final concentration per well) polysorbate-80 as described in Table 4 of M100-S16 (1, 2, 5, 7). Etest determinations for dalbavancin and vancomycin (the control agent) were performed using the manufacturer’s recommendations for incubation time, temperature, atmosphere, and inoculum concentrations on Mueller-Hinton agar (plus 5% sheep blood for testing of streptococci). MICs obtained by Etest and broth microdilution were generated for all 200 strains tested; a subset of 100 strains was tested concurrently using the agar dilution procedure.

All QC replicates (five) for each of the three QC strains were within the published ranges established by the CLSI (1,
In summary, the results from the dalbavancin Etest procedure demonstrated a high level of intermethod agreement (100% within ±2 log₂ dilution steps) with the reference broth microdilution procedure, whereas comparability with the agar dilution procedure was less acceptable, with a clear trend toward elevated MICs by the latter method. Utilization of the Etest method as an accurate alternative procedure to the broth microdilution procedure demonstrated a high level of intermethod agreement between standardized broth microdilution and agar dilution results presented here confirm the difficulties inherent in testing this agent using agar-based methodologies.

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REFERENCES


TABLE 1. Variation in dalbavancin and vancomycin MIC ratios obtained by comparing Etest (AB BIODISK) MICs with those from the reference broth microdilution method

<table>
<thead>
<tr>
<th>Antimicrobial and organism (no. tested)</th>
<th>Dalbavancin</th>
<th>Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of strains with the following Etest/broth microdilution MIC ratio:</td>
<td>No. of strains with the following Etest/agar dilution MIC ratio:</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>S. aureus (90)</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>CoNS (20)</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Enterococcus spp. (20)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>β-Streptococci (37)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Viridans group</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>streptococci (13)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>S. pneumoniae (20)</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Total (200)</td>
<td>8</td>
<td>32</td>
</tr>
</tbody>
</table>

2. Comparison of vancomycin (the control agent) Etest results with standardized broth microdilution and agar dilution results demonstrated a high level of agreement: 59.2% and 72.0% had identical MICs, and 98.5 and 99.0% of results were within ±1 log₂ dilution step, respectively. One coagulase-negative staphylococcus agar dilution result was 3 log₂ dilutions lower than the Etest result (Tables 1 and 2).

Comparison of Etest MIC results with those from the reference broth microdilution method for dalbavancin demonstrated very acceptable agreement: 92.5% and 100% of results were within ±1 log₂ dilution step, respectively (Table 1). Among strains in six groups tested, only the beta-hemolytic and viridans group streptococci produced a slight bias, with Etest MICs being minimally elevated (approximately 1/2 log₂ dilution). Susceptible and resistant subsets of S. aureus also gave comparable results by the two methods.

Etest MIC results were less comparable to those generated by the agar dilution method (10): 82% of results were within ±1 log₂ dilution step, although nearly all MICs (98%) were within ±2 log₂ dilutions. The bias seen with the agar dilution results for dalbavancin consisted of an approximately twofold increase in MICs above corresponding Etest values, a trend seen with all tested species or organism groups (Table 2). The results of a comparison of dalbavancin MICs by the broth microdilution and agar dilution methods were very similar to those for the comparison of Etest and agar dilution (9% identical, 73% within ±1 log₂ dilution step, and 95% within ±2 log₂ dilution steps [data not shown]). Agar dilution has not been proposed as a standard method for testing dalbavancin; the results presented here confirm the difficulties inherent in testing this agent using agar-based methodologies.