CUSTOMER INFORMATION SHEET

Etest Daptomycin (DPC) – technical variables that may cause discrepancies in MIC results

BACKGROUND

Cubist Pharmaceutical Inc. has informed AB BIODISK (bioMérieux) that several US laboratories have found clinical MRSA isolates that test non-susceptible by Etest Daptomycin (DPC). However, the Etest results (MIC >1.0 µg/mL) were not always confirmed by the reference broth microdilution method (MIC ≤1 µg/mL). Examination of Etest QC results of all manufactured lots (N=32) of Etest DPC since 2003 and evaluation of extended testing of recent lots of Etest DPC at bioMérieux SA has not shown any upward MIC shift. Consequently, users that may be seeing an increase in MIC values with Etest DPC, including observations of non-susceptible categorical results, are recommended to do the following.

1. Repeat Etest DPC testing using a different brand/lot of Mueller Hinton agar (MHA) plates than currently used.
2. When results with another MHA brand are the same, contact bioMérieux SA for a new Etest DPC lot for further testing.

We have examined our manufacturing processes and quality control procedures, and investigated the influence of calcium variations in MHA, inoculum variations and reading of results to try and identify potential sources of discrepancies and rectify problems that may be associated with various technical variables. This document (CIS 014) serves as a reminder of the importance of adhering to technical recommendations for MIC testing when using Etest Daptomycin.

EFFECTS OF CALCIUM ON DAPTOMYCIN MIC RESULTS

Daptomycin requires physiologic levels of free calcium (Ca²⁺) for expression of adequate activity. The reference CLSI® broth microdilution method (BMD) uses a final concentration of 50 µg/mL Ca²⁺ in Mueller Hinton broth. Etest is the only agar based method cleared by the FDA for daptomycin susceptibility testing. Etest DPC comprises a predefined gradient of daptomycin (0.016-256 µg/mL) overlaid with a constant level of calcium to achieve an equivalent of 40 µg/mL of Ca²⁺. Etest DPC is tested on plain MHA plates. Validation studies for Etest DPC versus BMD have shown that MHA plates with Ca²⁺ levels between 25 and 40 µg/mL were suitable for use with Etest DPC.

The bioavailable free Ca²⁺ levels in brands and batches of MHA powder and commercial MHA plates can vary significantly and affect Etest DPC results. Some of the clinical MRSA strains from the field that were found to be non-susceptible by Etest (>1.0 µg/mL) were susceptible by the reference method when tested at a daptomycin reference laboratory i.e. approximately 50% of these strains had MIC values of ≤1 µg/mL by BMD.

Table 1 summarises daptomycin MIC results for 38 of the MRSA strains received from the field. We compared more precise daptomycin BMD results (0.25 dilution increments) with Etest DPC results obtained using different brands of MHA plates and Etest lots.

MIC PRECISION AND ACCURACY

The absence of an intermediate category challenges all MIC methods since technical variables such as calcium levels, lot to lot variability in reagents, and MIC precision may affect various methods and give rise to discrepant results. When comparing BMD results based on serial 2-fold dilutions with BMD based on 0.25 dilution increments, we have found at least 25% of 2-fold values of 1 µg/mL to be >1 (1.25-1.5 µg/mL) by the more precise dilution. In the absence of a genotypic marker and/or clinical failure data to verify in vitro non-susceptibility to daptomycin, the precision and accuracy of MIC results that straddle the current daptomycin FDA/CLSI susceptible/non-susceptible breakpoints of ≤1/ >1 µg/mL are important to help minimise categorical errors of false non-susceptible or false susceptible results.

Etest DAPTOMYCIN METHOD

1. Prepare the inoculum suspension in saline and ensure that the turbidity does not exceed the 0.5 McFarland standard. When using small agar plates, press out extra fluid from the swab before streaking to avoid excess inoculum. Excessive growth on the agar can “hug” the strip and give falsely elevated MIC results.
2. Use only MHA plates with consistent and appropriate Ca²⁺ levels (25-40 µg/mL).
3. Perform quality control (QC) using both Enterococcus faecalis ATCC® 29212™ (1-4) and Staphylococcus aureus ATCC® 29213™ (0.25-1 µg/mL). Use of two QC strains allows verification of results within the susceptible range and around the breakpoint. When results are out of QC specifications or consistently skewed at the limits, repeat the test using a different brand of MHA.
4. See the trouble-shooting section for information regarding on-going investigations of QC results for S. aureus ATCC® 29213™.
5. Read different Etest MIC endpoint patterns using the following Etest reading guidelines (Figures 1-4).
Etest DPC reading guidelines:
When endpoints are clear-cut, read the MIC where the inhibition ellipse intersects the MIC scale. When different growth/inhibition patterns are observed, read as follows:

**Slim ellipse:** read the MIC where growth is inhibited at the bottom of the slim ellipse (Figure 1).

**Dip effect:** read the MIC at the end of the dip (Figure 2).

**Trailing effects:** read the MIC where the haze/microcolonies are inhibited (Figure 3).

**Microcolonies:** read where the microcolonies are inhibited (Figure 4).

Examples of Etest Daptomycin MIC (µg/mL) results for MRSA strains.

1. **S. aureus ATCC® 29213™**
   - Slim ellipse/sharp endpoint
   - Etest 0.25/BMD 0.38

2. **S. aureus MRSA 1795**
   - Dip effect; read down the dip
   - Etest 0.5/BMD 0.75

3. **S. aureus MRSA 1823**
   - Read where haze/microcolonies are inhibited
   - Etest 1/BMD 0.75

4. **S. aureus MRSA 1800**
   - Read where microcolonies are inhibited
   - Etest 1/BMD 0.75

ETEST DPC TROUBLE-SHOOTING

1. For MIC results of >1 µg/mL, retest with a different brand of MHA plate than the one currently used.
2. Check the inoculum density to ensure that the lawn of growth is semi-confluent and not excessively heavy. Repeat the test if heavy growth is observed.
3. Use the reading guidelines for Etest DPC and illustrations above (Figures 1-4).
4. Quality control and monitoring of results:
   - Monitor your QC and clinical results to quickly capture trends and shifts of MIC and/or categorical results.
     - **Upward shifts** - MHA calcium levels may be too low, agar plates too thick (depth > 4.5 mm), inoculum too heavy, reading of endpoints inappropriate and/or too conservative.
     - **Downward shifts** - MHA calcium levels too high, agar plates too thin (< 3.5 mm) and/or inoculum too low.
5. Use the recommended storage and handling instructions for the different Etest packages (Single Pack, Blister and Foam).
<table>
<thead>
<tr>
<th>Grouped-daptomycin MIC (BMD)</th>
<th>Number</th>
<th>BMD (1)</th>
<th>Etest DPC (4 lots; blister &amp; foam packs) (1 N 152)</th>
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<tbody>
<tr>
<td></td>
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<td>MHA (BD)</td>
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<tr>
<td>MRSA</td>
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<td>0.25-0.38</td>
<td>0.19-0.5</td>
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<td>(0.25)</td>
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<td>0.19-0.75</td>
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<td>(0.5)</td>
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<td>4</td>
<td>0.5-0.75</td>
<td>0.38-1</td>
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<td>(0.5)</td>
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<tr>
<td>MRSA</td>
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<td>(1)</td>
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<tr>
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<td>0.75-1.25</td>
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<td>(1)</td>
<td>(1)</td>
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<tr>
<td>hGISA</td>
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<td>2-2.5</td>
<td>1.5-2</td>
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<td>(2)</td>
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<td>(CLSI 1-4)</td>
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<tr>
<td>QC/MSSA</td>
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<td>(CLSI 0.125-1)</td>
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<tr>
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<td>(1.5)</td>
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Essential Agreement: MIC ± 1 dilution
Category Agreement: BMD vs. Etest

97% 92% 97%
86% 60% 88%

Notes:
1) All strains were tested in triplicate for each technical parameter. Value in ( ) is the mode.
2) To improve precision of reference results, BMD was performed with 0.25 dilution increments.
3) QC results for Staphylococcus aureus ATCC® 29213™ for BMD and Etest (regardless of Etest lots and calcium variations in MHA).

REFERENCES

Photos: bioMérieux SA

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