**Evaluation of the Oxoid Lefamulin 20 μg Antimicrobial Susceptibility Testing (AST) Disc Against the Predicate Lefamulin 20 μg AST Disc**

**A. J. Lovatt,1 N. M. Holliday,2 C. C. Knapp,2 E. Best1, K. Simpson3, J. Lindley4, D. Shortridge5, S. Paukner6

1Thermo Fisher Scientific, Basingstoke, UK; 2Thermo Fisher Scientific, Cleveland, OH, USA; 3JMI laboratories, North Liberty, IA, USA; 4Nabriva Therapeutics, Vienna, AT

**ABSTRACT**

**Background**

Lefamulin is the first pleuromutilin developed for both intravenous and oral administration in humans with activity against Gram-positive, fastidious Gram-negative and atypical respiratory bacteria. Lefamulin is indicated for the treatment of adults with community-acquired pneumonia (CAP) caused by the following organisms: Streptococcus pneumoniae, Staphylococcus spp., and Haemophilus influenzae. Recommendations for Clinical and Laboratory Standards Institute (CLSI) quality control (QC) organ isolates were tested against 2 lots of Oxoid lefamulin discs and 1 lot of Hardy Disk discs. All isolates were tested in accordance with CLSI M2-A11 and CLSI M31-A10 using FDA-cleared Mueller Hinton agar (Thermo Scientific™ Remel™ MHA supplied by Thermo Fisher). MHA+5% sheep blood for S. aureus, Staphylococcus spp. and Haemophilus Test Medium (HTM) for Haemophilus influenzae. All testing was conducted by JMI laboratories (North Liberty, IA, USA).

**Methods**

The Oxoid lefamulin discs and Hardy Disk lefamulin discs were tested simultaneously against 424 clinical and challenge isolates and 15 reproducibility isolates including Streptococcus pneumoniae, Staphylococcus spp., and Haemophilus influenzae. Recommended Clinical and Laboratory Standards Institute (CLSI) quality control (QC) organ isolates were tested against 2 lots of Oxoid lefamulin discs and 1 lot of Hardy Disk discs. All isolates were tested in accordance with CLSI M2-A11 and CLSI M31-A10 using FDA-cleared Mueller Hinton agar (Thermo Scientific™ Remel™ MHA supplied by Thermo Fisher). MHA+5% sheep blood for S. aureus, Staphylococcus spp. and Haemophilus Test Medium (HTM) for Haemophilus influenzae. All testing was conducted by JMI laboratories (North Liberty, IA, USA).

**Results**

Overall, a categorical agreement of 99.2% was achieved when the Oxoid lefamulin disc was compared to the predicate device with no minor and no major discrepancies and 3 very major discrepancies observed. Lefamulin exhibited disc zone sizes of 12-40 mm for S. pneumoniae (mode 27 mm), 6-41 mm for S. aureus (mode 27 mm) and 18-38 mm for S. pneumoniae (mode 24 mm). All isolates showed 100% reproducibility with each other and between-reader by calculating the percent of results which were less than ±3 mm of the modal value. QC results were within the stated limits 99.7% of the time for each batch and reader.

**Conclusions**

The Oxoid lefamulin disc compared to the Hardy Disk demonstrated an equivalent level of performance. The high categorical agreement obtained by the Oxoid lefamulin disc suggests this is an acceptable method for antimicrobial susceptibility testing of lefamulin.

**INTRODUCTION**

Lefamulin (Figure 1) is the first pleuromutilin developed for both intravenous and oral administration in humans with activity against Gram-positive, fastidious Gram-negative and atypical respiratory bacteria. In this study, we were interested to evaluate the performance and reproducibility of the new Thermo Fisher® Oxoid lefamulin 20 μg (Thermo Fisher Scientific) Lefamulin for Antimicrobial Susceptibility Testing (AST) disc against a predicate device, the Food and Drug Administration (FDA) cleared lefamulin 20 μg HardyDisk™ (Hardy diagnostics, Santa Maria, CA).

**MATERIALS AND METHODS**

**Clinical and Challenge isolates**

One lot of Oxoid lefamulin discs were tested against one lot of Hardy lefamulin discs for a total of 424 clinical and challenge isolates (375 isolates for indicated organisms, 49 isolates for non-indicated organisms) including Staphylococcus spp., Staphylococcus aureus, and Haemophilus influenzae (Table 1). The non-identified organisms were tested for information purposes only. All isolates were shared between laboratories (approximately 141 isolates each) to represent three testing sites which were then analysed using breakpoints set by the FDA (Figure 2).

**Reproducibility**

Two lots of Oxoid lefamulin discs were tested and read by three individuals against 15 indicated and on-scale reproducibility isolates over a 3-day testing period to generate a total of 270 data points.

**Quality control**

Quality control strains from the American Type Culture Collection (ATCC) were tested against the Oxoid lefamulin discs and Hardy Disk lefamulin discs to ensure all AST discs were within the QC limits. At least 20% of each lot of Oxoid lefamulin discs were tested per individual (3 individuals) to represent 3 testing sites.

**The QC zone size limits for lefamulin 20 μg AST discs are as follows:**

- **Staphylococcus aureus (ATCC® 29213)**
  - QC limit: 26-32 mm

- **Streptococcus pneumoniae (ATCC® 29619)**
  - QC limit: 24-30 mm

**RESULTS**

**Quality Control**

 QC results were within the stated limits for Staphylococcus pneumoniae and Haemophilus influenzae 100% of the time for each lot of Oxoid lefamulin discs. QC results were within specification for Staphylococcus aureus 99.7% of the time for lot 1 and 100% for lot 2.

**Reproducibility**

All data showed 100% reproducibility for both lots of lefamulin Oxoid discs, within-reader and between-reader. This was calculated as the percent of results which were less than ±3 mm of the modal value. The summary is shown in Table 3.

**Conclusions**

The Oxoid lefamulin disc is an acceptable method for AST testing having also recently been FDA-cleared for the testing of Gram-positive and fastidious Gram-negative bacteria.

**REFERENCES**


**Figure 2. Lefamulin zone of inhibition (Streptococcus aureus)**

**Table 1. Number of isolates tested during the study.**

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Number Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical isolates</td>
<td>320</td>
</tr>
<tr>
<td>Challenge isolates</td>
<td>94</td>
</tr>
<tr>
<td>Reproducibility isolates</td>
<td>15</td>
</tr>
<tr>
<td>ATCC Quality Control Strains</td>
<td>3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>442</td>
</tr>
</tbody>
</table>

**Table 2. FDA-break points for Lefamulin.**

<table>
<thead>
<tr>
<th>Organism(s)</th>
<th>Zone Diameter Interpretive Criteria (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pneumoniae</td>
<td>≥23</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>≥17</td>
</tr>
</tbody>
</table>

**Table 3. Summary of the reproducibility of Oxoid Lefamulin discs between 2 lots and 3 individuals.**

<table>
<thead>
<tr>
<th>Reproducibility between disc lots</th>
<th>Reproducibility between individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1 Lot 2 All Lots Individual 1 Individual 2 Individual 3 All Individuals</td>
<td>100% 100% 100% 100% 100% 100% 100%</td>
</tr>
</tbody>
</table>

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