Amie Lovatt
Scientist II (510(k) specialist)
Thermo Fisher Scientific
Basingstoke, UK
amie.lovatt@thermofisher.com

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.Best[1], K.Simpson[3], J.Lindley[3], D.Shortridge[3], S.Paukner[4].
[1]Thermo Fisher Scientific, Basingstoke, UK; [2]Thermo Fisher Scientific, Cleveland, OH, USA; [3] JMI laboratories, North Liberty, IA, USA; [4] Nabriva 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 aureus, Haemophilus influenzae, Legionella pneumophila, Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*. A study was conducted to evaluate the performance and reproducibility of the new Thermo Fisher™ Oxoid™ lefamulin 20 µg (Thermo Fisher Scientific, Basingstoke, UK) 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).

Methods
The Oxoid lefamulin discs and Hardy lefamulin discs were tested simultaneously against 424 clinical and challenge isolates and 15 reproducibility isolates including *Streptococcus* spp., *Staphylococcus* spp. and *Haemophilus influenzae*. Recommended Clinical and Laboratory Standards Institute (CLSI) quality control (QC) organisms were tested daily against 2 lots of Oxoid lefamulin discs and 1 lot of Hardy lefamulin discs. All isolates were tested in accordance with CLSI M02[1,2]/M100[3] using FDA-cleared Mueller Hinton agar (Thermo Scientific™ Remel ™ MHA supplied by Thermo Fisher), MHA+5% sheep blood for *Streptococcus* 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 *H. influenzae* (mode 27 mm), 6-41 mm for *S. aureus* (mode 31 mm) and 18-38 mm for *S. pneumonaie* (mode 24 mm). All data showed 100% reproducibility within-reader and between-reader by calculating as 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 HardyDisk 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. This novel compound acts by binding to the peptidyl transferase center (PTC) on the bacterial ribosome to interfere with the interaction of protein production, therefore resulting in the inhibition of bacterial proteins and bacterial growth. Lefamulin is indicated for the treatment of adults with community acquired pneumonia (CAP) caused by *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Legionella pneumophila, Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*.

An *in vitro* study was conducted by JMI laboratories to evaluate the performance and reproducibility of the new lefamulin 20 µg Oxoid AST disc against a predicate device, lefamulin HardyDisk 20 µg.

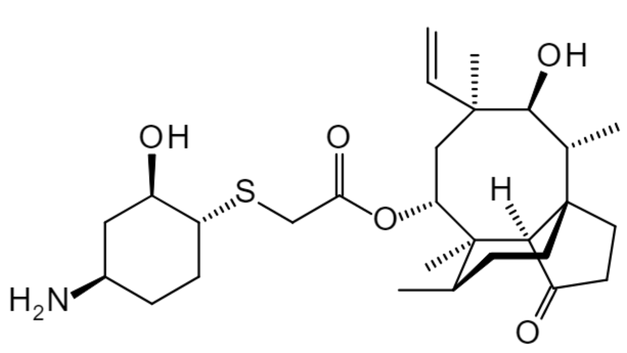

Figure 1. The chemical structure of lefamulin.

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 *Streptococcus* spp., *Staphylococcus* spp. and *Haemophilus influenzae* (Table 1). The non-indicated organisms were tested for information purposes only. All isolates were shared between three individuals (approximately 141 isolates each) to represent three testing sites which were then analysed using breakpoints set by the FDA[4,5] (Table 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 daily for both Hardydiscs and Oxoid discs alongside clinical, challenge and reproducibility isolates to ensure all AST discs were within the QC limits. At least 20 replicates of the quality control strains 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® 25923)
  QC limit: 26-32 mm
- *Streptococcus pneumoniae (*ATCC® 49619[a])
  QC limit: 19-27 mm
- *Haemophilus influenzae (*ATCC® 49247)
  QC limit: 22-28 mm

Colony counts were performed on inoculums for at least 10% of clinical/challenge isolates and all QC and reproducibility isolates.

All isolates were tested according to the manufacturer's instructions and the CLSI M02[1,2]/M100[3] using FDA-cleared Mueller Hinton agar (Thermo Scientific™ Remel™ MHA supplied by Thermo Fisher), MHA+5% sheep blood for *Streptococcus* spp. and Haemophilus Test Medium (HTM) for *Haemophilus influenzae.* All testing was conducted by JMI laboratories.

Table 1. Number of isolates tested during the study.

Isolates	Number Tested
Clinical Isolates	330
Challenge Isolates	94
Reproducibility Isolates	15
ATCC Quality Control Strains	3
TOTAL	442

Table 2. FDA break points for Lefamulin[4].

Organism(s)	Zone Diameter Interpretive Criteria (mm)		
	S	I	R[a]
Methicillin-susceptible *Staphylococcus aureus* (MSSA)	≥23	-	-
S. pneumoniae	≥17	-	-
H. influenzae	≥17	-	-

S= Susceptible, I= Intermediate, R= Resistant.
[a] No resistance breakpoints were established, due to the absence of resistant isolates among these species.

RESULTS

Quality Control
QC results were within the stated limits for *Streptococcus 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.

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

Reproducibility between disc lots			Reproducibility between individuals			
Lot 1	Lot 2	All Lots	Individual 1	Individual 2	Individual 3	All Individuals
100%	100%	100%	100%	100%	100%	100%

Clinical and Challenge isolates
The categorical agreement of the Oxoid lefamulin disc was determined by the data shown in Table 4.

Table 4. Analysis of Lefamulin Oxoid disc vs. HardyDisk for indicated species.

	Number of isolates tested	Number of isolates in categorical agreement (CA)	Number of discrepancies
Organism			
***Staphylococcus aureus* (MSSA)**			
Clinical	100	100	0
Challenge	25	25	0
Combined	125	125	0
Haemophilus influenzae			
Clinical	100	99	1
Challenge	25	23	2
Combined	125	122	3
Streptococcus pneumoniae			
Clinical	100	100	0
Challenge	25	25	0
Combined	125	125	0

The indicated species tested had the following categorical agreement (CA):

- *Staphylococcus aureus* (MSSA), 100%
- *Streptococcus pneumoniae*, 100%
- *Haemophilus influenzae*, 97.6%

RESULTS Cont.

The overall categorical agreement achieved for all indicated isolates tested in this study was 99.2%, when the Oxoid lefamulin disc was compared to the predicate device. The FDA acceptance criteria for categorical agreement is > 89.9%.
No minor, no major discrepancies and only 3 very major discrepancies with *H. influenzae* were observed during the study which correlates to a 0.8% very major rate overall. In instances where a very major discrepancy was observed the Oxoid lefamulin disc produced results 1-3 mm larger than the predicate device, leading to a classification of susceptible instead of a non-susceptible result. The FDA acceptance criteria for very major errors is ≤1.5%.
Lefamulin exhibited disc zone sizes of 12-40 mm for *H. influenzae* (mode 27 mm), 6-41 mm for S. aureus (mode 31 mm) and 18-38 mm for *S. pneumonaie* (mode 24 mm).

Forty-nine non-indicated species were tested alongside indicated isolates for information purposes only, shown in Table 4. All zone of inhibition sizes for the lefamulin Oxoid disc were within 2 mm of the predicate result.

Table 4. Analysis of Lefamulin Oxoid disc vs. Hardydisc for non-indicated species.

	Number of isolates			Identical to Predicate (%)			
Organism	**Clinical**	**Challenge**	**Total**	**± 0 mm**	**± 1 mm**	**± 2mm**	**± 3mm**
Methicillin-resistant *Staphylococcus aureus* (MRSA)	30	10	40	28	75	100	100
Streptococcus agalactiae	0	9	9	100	100	100	100

All colony counts were in the region of 1-2 x10^8 CFU/mL for all QC and reproducibility isolates and 10% of the clinical and challenge isolates.

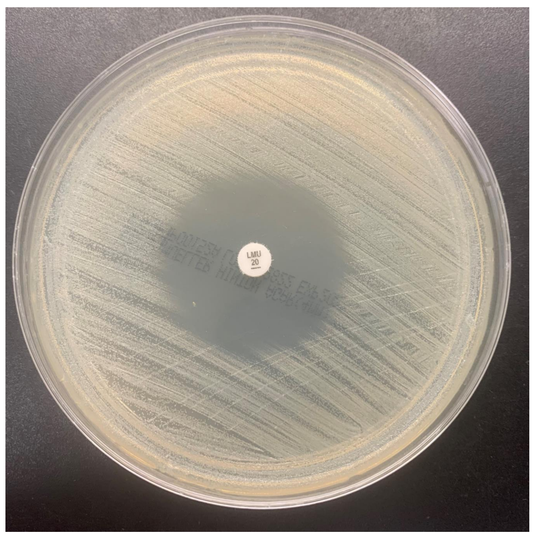

Figure 2. lefamulin zone of inhibition (*Staphylococcus aureus).*

CONCLUSION

This study validates that the Oxoid Lefamulin 20 µg AST disc has an equivalent level of performance compared to the FDA cleared, lefamulin Hardydisk. The Oxoid Lefamulin AST 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

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[5] FDA *Guidance for Industry and FDA Class II Special Controls Guidance Document:* Antimicrobial Susceptibility Test (AST) Systems, August 28, 2009.