

# A Multi-Site Study Comparing a Commercially Prepared Dried MIC Susceptibility System to the CLSI/ISO Broth Microdilution Method for FDA Approved Delafloxacin (BAXDELA™) using Gram-Negative Non-Fastidious Organisms

\*N. M. Holliday<sup>1</sup>, C. C. Knapp<sup>1</sup>, S. M. Andrus<sup>1</sup>, S.B. Killian<sup>1</sup>, T.C. Lewis<sup>1</sup>, J.M. Lindley<sup>2</sup>, J. M. Streit<sup>2</sup>, B.J. Olson<sup>3</sup>, T.R. Fritsche<sup>3</sup>, J.W. Decousser<sup>4</sup>, E. Scopes<sup>5</sup>, A.M. Leonte<sup>5</sup>, S. McCurdy<sup>6</sup>

<sup>1</sup>Thermo Fisher Scientific, Cleveland, OH; <sup>2</sup>JMI Laboratories, North Liberty, IA; <sup>3</sup>Marshfield Clinic, Marshfield, WI; <sup>4</sup>Laboratoire de Bactériologie – Hygiène Chu Henri, Mondor, France; <sup>5</sup>Thermo Fisher Scientific, Basingstoke, UK; <sup>6</sup>Melinta Therapeutics, New Haven, CT.

## ABSTRACT

**Background:** Delafloxacin (DLX) (BAXDELA™) (Melinta Therapeutics, New Haven, CT), is a fluoroquinolone antibacterial that has been FDA approved for the treatment of acute bacterial skin and skin structure infections (ABSSSI) caused by gram-negative and gram-positive organisms. A four site evaluation was performed to determine the accuracy and reproducibility of DLX susceptibility testing against gram-negative non-fastidious organisms using the Thermo Scientific™ Sensititre™ 18-24 hour dried MIC susceptibility system (Thermo Fisher Scientific, Cleveland, OH) compared with the CLSI (M07)/ISO 20776-1/ISO 20776-2 (CLSI/ISO) reference broth microdilution method (BMD). Both auto (Optiread™) and manual read methodologies were employed.

**Materials and Methods:** DLX (0.004-8 µg/mL) was tested against 304 recent clinical isolates, 75 challenge isolates and 11 reproducibility isolates. These isolates consisted of 132 *Escherichia coli* (including ESBL+/-, and KPC), 139 *Klebsiella pneumoniae* (including ESBL+/-, and KPC), 58 *Enterobacter cloacae*, and 61 *Pseudomonas aeruginosa*. The Sensititre dried MIC susceptibility panels were inoculated per manufacturer's instructions. BMD was performed per CLSI/ISO guidelines. Recommended CLSI quality control (QC) organisms were tested daily and all results were within the published QC ranges.

**Results:** Comparisons of the indicated gram-negative non-fastidious organisms MIC results on the FDA cleared Sensititre system for automated and manual reads to the CLSI/ISO BMD MICs resulted in 98.1% and 98.9% essential agreements (EA; +/- 1 log<sub>2</sub> dilution), respectively. Overall agreement for the reproducibility (+/- 1 log<sub>2</sub> dilution of the modal MIC) using automated and manual reads was 99.7% and 99.5%, respectively.

**Conclusions:** The results for DLX indicate that the Sensititre dried MIC susceptibility system for all clinical and challenge gram-negative non-fastidious organisms gave reliable results using either the automated or manual read methods compared to the reference CLSI/ISO BMD. The FDA has determined that DLX is substantially equivalent for the indications of *E. coli*, *E. cloacae*, *K. pneumoniae*, and *Ps. aeruginosa* on the Sensititre™ dried MIC susceptibility system and has been cleared for *in vitro* diagnostic use.

**INTRODUCTION** Delafloxacin (Figure 1.) is a novel, FDA approved, fluoroquinolone that inhibits topoisomerase IV and DNA gyrase enzymes in the treatment of acute bacterial skin and skin structure infections (ABSSSI) caused by gram-negative non-fastidious organisms. This *in vitro* multi-site comparison study was performed to evaluate the performance of delafloxacin on the commercially manufactured Sensititre® 18-24 hour susceptibility system, for both automated and manual reads, compared against the Clinical Laboratory Standards Institute (CLSI) reference broth microdilution (BMD) method (M07/M100) and ISO 20776-1 (BMD). To establish equivalency between the two methods, a 4 lab clinical study was conducted, and the MIC results obtained using the Sensititre dried plate technology were compared to the MIC results from the CLSI M07 frozen reference plate.

## MATERIALS AND METHODS

●The Sensititre 18-24 hour MIC susceptibility system (Thermo Fisher Scientific, Oakwood Village, OH) is an *in vitro* diagnostic product for clinical susceptibility testing of both fastidious and non-fastidious organisms. delafloxacin was tested against: (Table 1.)

- 304 recent clinical isolates across the four sites
- 75 challenge isolates at a single testing site
- 11 reproducibility isolates at each site (tested in triplicate over a 3 day testing period)
- 2 Quality Control Strains (ATCC)

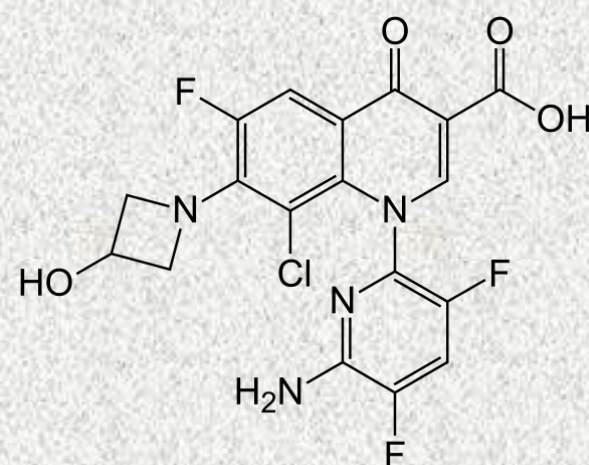


Figure 1. Chemical Structure of Delafloxacin

## MATERIALS AND METHODS Cont.

● Colony Counts and purity plates were performed on the inocula of the Clinical, Challenge, Reproducibility and QC strains on each day of testing.

● Each isolate was tested using a:

- Dried Sensititre 18–24 susceptibility plate containing delafloxacin (0.004-8µg/ml). The dried plates were set up and tested by both automated and manual reading methodologies according to the manufacturer's instructions.

- CLSI reference broth microdilution plate was prepared and tested on each isolate according to the current Clinical Laboratory Standards Institute standard method.

Table 1. Organisms Tested	Number Tested
Clinical Isolates (4 sites)	304
CDC Challenge Isolates (one site)	75
Reproducibility Isolates (4 sites) (3 x day for 3 days)	11 (396)
ATCC Quality Control Strains (20 replicates of each strain at 4 sites)	2 (160)
<b>TOTAL</b>	<b>935</b>

### Quality Control

● Recommended CLSI quality control (QC) organisms were tested daily and were within the CLSI expected QC ranges.

● Colony counts were performed and fell within expected ranges  
Reference 2-8X10<sup>5</sup>, Sensititre 5X10<sup>4</sup>-5X10<sup>5</sup>

Table 2. Quality Control Strains	CLSI QC Ranges (µg/ml)
<i>Escherichia coli</i> ATCC 25922	0.008-0.03
<i>Pseudomonas aeruginosa</i> ATCC 27853	0.12-0.5

## Results

Essential agreement for delafloxacin on the Sensititre susceptibility plate compared to the reference microdilution plate was calculated for each read method (Auto and Manual ) using the +/- one log<sub>2</sub> dilution standard. Essential agreement rates are shown for gram-negative non-fastidious isolates in **Tables 3 and 4.**

### Clinical Isolates and Challenge Organisms

The overall essential agreement for delafloxacin within ±1 log<sub>2</sub> dilution was **98.9%** for the manual method and **98.1%** for the auto read method.

### Inter-laboratory Reproducibility

Reproducibility testing results for delafloxacin within ±1 log<sub>2</sub> dilution from the modal MIC was **99.7%** for the auto read method and **99.5%** for the manual read method (**Table 5**).

## RESULTS Cont.

**Table 3. Summary Data and % Essential Agreement of gram-negative non-fastidious Clinical and Challenge Isolates Using the Manual Read Method**

The overall essential agreement for delafloxacin within +/- one log<sub>2</sub> dilution, was **98.9%** for the manual read method

### Combined Total Isolates

Delafloxacin	Number of Isolates		Essential Agreement		% Essential Agreement	
	All	Evaluable	Total	Evaluable	Total	Evaluable
<b>Organism Group</b>						
<i>Escherichia coli</i>	127	120	124	117	97.6%	97.5%
<i>Klebsiella pneumoniae</i>	135	109	134	108	99.3%	99.1%
<i>Enterobacter cloacae</i>	57	53	57	53	100.0%	100.0%
<i>P. aeruginosa</i>	60	51	60	51	100.0%	100.0%
<b>Total</b>	<b>379</b>	<b>333</b>	<b>375</b>	<b>329</b>	<b>98.9%</b>	<b>98.8%</b>

**Table 4. Summary Data and % Essential Agreement of gram-negative non-fastidious Clinical and Challenge Isolates Using the Auto Read Method**

The overall essential agreement for delafloxacin within +/- one log<sub>2</sub> dilution, was **98.1%** for the auto read method

### Combined Total Isolates

Delafloxacin	Number of Isolates		Essential Agreement		% Essential Agreement	
	All	Evaluable	Total	Evaluable	Total	Evaluable
<b>Organism Group</b>						
<i>Escherichia coli</i>	127	120	123	116	96.9%	96.7%
<i>Klebsiella pneumoniae</i>	135	109	134	108	99.3%	99.1%
<i>Enterobacter cloacae</i>	57	53	55	51	96.5%	96.2%
<i>P. aeruginosa</i>	59	49	59	49	100.0%	100.0%
<b>Total</b>	<b>378</b>	<b>331</b>	<b>371</b>	<b>324</b>	<b>98.1%</b>	<b>97.9%</b>



## RESULTS Cont.

**Table 5. Inter-laboratory Reproducibility % Essential Agreement ±1 log<sub>2</sub> dilution from the Modal Value**

Delafloxacin	Auto Read	Manual Read
Between-site total isolates tested	396	396
Between-site isolates within +/- 1 well from mode	395	394
Between-site reproducibility ratio	395	394
Between-site reproducibility %	<b>99.7%</b>	<b>99.5%</b>
Total essential agreement	395/396	394/396
<b>Essential agreement %</b>	<b>99.7%</b>	<b>99.5%</b>

## CONCLUSIONS

This study validates that the Sensititre 18–24 hour susceptibility system (both auto read and manual read) demonstrated an equivalent level of performance compared to the CLSI M07/M100 reference broth microdilution plate when testing FDA approved delafloxacin against gram-negative non-fastidious clinical and challenge isolates. This study suggests that this is an acceptable method for susceptibility testing of delafloxacin.

## REFERENCES

Clinical and Laboratory Standards Institute. 2015. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-tenth edition*. Approved document M07-A10. Wayne, PA: CLSI.

Clinical and Laboratory Standards Institute. 2018. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-seventh Informational Supplement M100-S28*. Wayne, PA: CLSI.

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

*Clinical laboratory testing and in vitro diagnostic test systems - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices - Part 1: Reference method for testing the in vitro activity of antimicrobial agents against rapid I growing aerobic bacteria involved in infectious diseases* (ISO 20776-1 :2006).

© 2018 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.