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**Nicole M. Holliday**
**Supervisor AST/Pharma R&D**
**Thermo Fisher Scientific**
**Oakwood Village, OH 44146**
**1-800-871-8909 ext. 322-4319**
**nicole.holliday@thermofisher.com**

# 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__[1], C. C. Knapp[1], S. M. Andrus[1], S.B. Killian[1], T.C. Lewis[1], J.M. Lindley[2],J. M. Streit[2], B.J. Olson[3], T.R. Fritsche[3], J.W. Decousser[4], E. Scopes[5], A.M. Leonte[5], S. McCurdy[6]
[1]Thermo Fisher Scientific, Cleveland, OH; [2]JMI Laboratories, North Liberty, IA; [3]Marshfield Clinic, Marshfield, WI; [4]Laboratoire de Bactériologie – Hygiène Chu Henri, Mondor, France; [5]Thermo Fisher Scientific, Basingstoke, UK; [6]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 $\log_2$ dilution), respectively. Overall agreement for the reproducibility (+/- 1 $\log_2$ 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 CLIS/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)

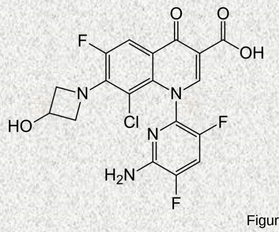


Figure 1. Chemical Structure of Delafloxacin

## 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) |
| **TOTAL** | **935** |

### 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 2-8X$10^5$, Sensititre 5X$10^4$-5X$10^5$

| Table 2. Quality Control Strains | CLSI QC Ranges (µg/ml) |
|---|---|
| *Escherichia coli* ATCC 25922 | 0.008-0.03 |
| *Pseudomonas aeruginosa* 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 $\log_2$ 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 $\log_2$ 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 $\log_2$ 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 $\log_2$ dilution, was 98.9% for the manual read method**

**Combined Total Isolates**

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

**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 $\log_2$ dilution, was 98.1% for the auto read method**

**Combined Total Isolates**

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


## RESULTS Cont.

**Table 5. Inter-laboratory Reproducibility % Essential Agreement ±1 $\log_2$ 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 % | **99.7%** | **99.5%** |
| Total essential agreement | 395/396 | 394/396 |
| **Essential agreement %** | **99.7%** | **99.5%** |

## 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.

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*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).