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

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## 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 *Streptococcus* spp., using the Thermo Scientific™ Sensititre™ 18-24 hour dried MIC susceptibility system (Thermo Fisher Scientific, Cleveland, OH) compared with the CLSI (M07) reference broth microdilution method (BMD). Both auto (Optiread™) and manual read methodologies were employed.
**Materials and Methods:** DLX (0.0005-8 µg/mL) was tested against 258 recent clinical isolates, 59 challenge isolates and 10 reproducibility isolates. Microorganisms tested included 124 *Streptococcus pyogenes*, 131 *Streptococcus agalactiae*, 67 *Streptococcus anginosus* Group, and 5 other *Streptococcus spp.* (reproducibility). The Sensititre dried MIC susceptibility plates were inoculated per manufacturer's instructions. BMD was performed per CLSI guidelines. Recommended CLSI quality control (QC) organisms were tested daily and all results were within the published QC ranges.
**Results:** Comparisons of the indicated *Streptococcus* spp. MIC results on the FDA cleared Sensititre system for automated and manual reads to the CLSI BMD MICs resulted in 99.1% and 99.7% essential agreements (EA; +/- 1 $\log_2$ dilution), and 99.1% and 100% categorical agreements respectively. Overall agreement for the reproducibility (+/- 1 $\log_2$ dilution of the modal MIC) using automated and manual reads was 99.6% and 97.9%, respectively.
**Conclusions:** The results of this DLX study on Sensititre™ dried MIC susceptibility system (both automated and manual read) demonstrated an equivalent level of performance compared to the CLSI BMD when testing DLX against *Streptococcus* spp. The FDA has determined that DLX is substantially equivalent for the indications of *Streptococcus pyogenes, Streptococcus agalactiae,* and *Streptococcus anginosus* Group 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 enzymes and DNA gyrase in the treatment of acute bacterial skin and skin structure infections (ABSSSI). 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). 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 obtained from the CLSI M07 frozen reference plate.

## MATERIALS AND METHODS

- The Sensititre 18-24 hour MIC 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.)

- 258 recent clinical isolates across the four sites
- 59 challenge isolates at a single testing site
- 10 reproducibility isolates at each site (tested in triplicate over a 3 day testing period)
- 1 Quality Control Strain (ATCC)

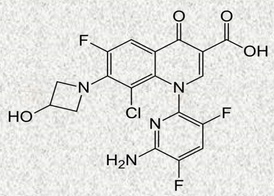


Figure 1. Chemical Structure of Delafloxacin

## 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 (0.0005-8µg/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) | 258 |
| **CDC Challenge Isolates** (one site) | 59 |
| **Reproducibility Isolates** (4 sites) (3 x day for 3 days) | 10 (360) |
| **ATCC Quality Control Strains** (20+ replicates of each strain at 4 sites) | 1 (90) |
| **TOTAL** | **767** |

### 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 Strain** | **CLSI QC Ranges** (µg/ml) |
|---|---|
| *Streptococcus pneumoniae* ATCC 49619 | 0.004-0.015 |

## 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 *Streptococcus* spp. in **Tables 3 and 4**.

**Clinical Isolates and Challenge Organisms**

The overall essential agreement for delafloxacin within ±1 $\log_2$ dilution was **99.7%** for the manual method and **99.1%** for the auto read method.

**Inter-laboratory Reproducibility**

Reproducibility testing results for delafloxacin within ±1 $\log_2$ dilution from the modal MIC was **100.0%** for the auto read method and **99.4%** for the manual read method **(Table 5)**.

## RESULTS Cont.

**Table 3. Summary Data and % Essential Agreement of *Streptococcus* spp. Clinical and Challenge Isolates Using the Manual Read Method**

**The overall essential agreement for delafloxacin within +/- one $\log_2$ dilution, was 99.7% for the manual read method**

**Combined Total Isolates**

| **Delafloxacin** | Number of Isolates | | Essential Agreement | | % Essential Agreement | |
|---|---|---|---|---|---|---|
| **Organism Group** | All | Evaluable | Total | Evaluable | Total | Evaluable |
| *Streptococcus pyogenes* | 123 | 123 | 123 | 123 | 100.0% | 100.0% |
| *Streptococcus agalactiae* | 129 | 129 | 129 | 129 | 100.0% | 100.0% |
| *Streptococcus anginosus* Group | 65 | 65 | 64 | 64 | 98.5% | 98.5% |
| **Total** | **317** | **317** | **316** | **316** | **99.7%** | **99.7%** |

**Table 4. Summary Data and % Essential Agreement of *Streptococcus* spp. Clinical and Challenge Isolates Using the Auto Read Method**

**The overall essential agreement for delafloxacin within +/- one $\log_2$ dilution, was 99.1% for the auto read method**

**Combined Total Isolates**

| **Delafloxacin** | Number of Isolates | | Essential Agreement | | % Essential Agreement | |
|---|---|---|---|---|---|---|
| **Organism Group** | All | Evaluable | Total | Evaluable | Total | Evaluable |
| *Streptococcus pyogenes* | 123 | 123 | 122 | 1122 | 99.2% | 99.2% |
| *Streptococcus agalactiae* | 129 | 129 | 128 | 128 | 99.2% | 99.2% |
| *Streptococcus anginosus* Group | 65 | 65 | 64 | 64 | 98.5% | 98.5% |
| **Total** | **317** | **317** | **314** | **314** | **99.1%** | **99.1%** |


## 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 | 360 | 360 |
| Between-site isolates within +/- 1 well from mode | 360 | 358 |
| Between-site reproducibility ratio | 360 | 358 |
| Between-site reproducibility % | **100.0%** | **99.4%** |
| Total essential agreement | 360/360 | 358/360 |
| **Essential agreement %** | **100.0%** | **99.4%** |

## 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 *Streptococcus* spp. 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.