Evaluation of Thermo Scientific™ Sensititre™ system for MIC determination of cephalosporins with EUCAST breakpoints for Enterobacteriaceae, Acinetobacter and Pseudomonas


OVERVIEW
Purpose: To evaluate the performance of Thermo Scientific Sensititre susceptibility MIC plates (Thermo Fisher Scientific) for antimicrobial susceptibility testing of Enterobacteriaceae, Acinetobacter and Pseudomonas, using automatic (Thermo Scientific™ Sensititre™ OptiRead™ System) and manual (Thermo Scientific™ Sensititre™ Vizion™ System) reading methods, according to ISO 20776-2 guidelines for Antimicrobial Susceptibility Testing (AST) as interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

Methods: Enterobacteriaceae, Acinetobacter and Pseudomonas clinical isolates were tested on Sensititre susceptibility MIC plates containing cephalexin, cefuroxime, cefixime, cefpodoxime, cefotaxime and cefepime.

Results: Sensititre susceptibility MIC plates showed >91% essential agreement and categorical agreement with the broth microdilution reference method for all cephalosporins tested.

INTRODUCTION
The Thermo Scientific Sensititre System is a leader in antimicrobial susceptibility and identification testing, offering flexible, customizable testing options to accommodate laboratories of all sizes. The Sensititre System consists of 96-well microtitre plates containing dilutions of antimicrobials dried in individual wells (see figure 1) (available in both standard and custom formats), as well as instrumentation to enable inoculation and interpretation (see figure 2). The Sensititre System utilizes true MIC results rather than extrapolated (MIC) results and offers flexible, customizable testing options to accommodate formularies and laboratories of all sizes conducting antimicrobial susceptibility and identification (AST/ID) testing.

FIGURE 1. Thermo Scientific Sensititre Susceptibility MIC susceptibility plate

METHODS
Reproducibility
Reproducibility testing of 10 Gram negative isolates in triplicate over three days was performed on the Sensititre susceptibility MIC plates.

Clinical isolate testing
A total of 390 clinical isolates (including 54 Acinetobacter, 271 Enterobacteriaceae and 65 Pseudomonas) were tested in total. The Sensititre susceptibility MIC plate was inoculated and tested according to manufacturer’s instructions: plates were read using both the Sensititre Vizion (manual read) and Sensititre OptiRead (automated read); results were interpreted using the Thermo Scientific™ Sensititre™ SWIN™ Software system. The broth microdilution reference method was performed according ISO 20776-1:2006, and read using the Sensititre Vizion (manual read). Frozen broth microdilution plates were sourced from Thermo Fisher Scientific, Oakwood Village, OH, USA.

Quality control
Recommended quality control (QC) organisms were tested daily on the Sensititre susceptibility MIC, and broth microdilution plates. Purity checks and colony counts were performed daily on the broth microdilution reference method according to ISO 20776-1:2006. Purity checks were conducted daily on the Sensititre susceptibility MIC plates and colony counts performed periodically according to manufacturer’s instructions.

FIGURE 2. Thermo Scientific Sensititre System

RESULTS
Data Analysis
Using EUCAST breakpoints, essential agreement (EA), categorical agreement (CA) and discrepancy rates were calculated according to ISO 20776-2:2007. The ISO 20776-2:2007 acceptance criteria are ≥90% EA, ≥90% CA and a very major discrepancy (VMD) rate of ≤3%. In instances where the testing does not include a significant number of resistant isolates the VMD may be greater than 3%, as the calculation is based on the number of resistant isolates. If the VMD rate is >3% but the EA is ≥97% the device is considered to have acceptable accuracy. Minor discrepancies (mD) were also reported.

RESULTS
Reproducibility
Intra- (daily) and inter- (between days) reproducibility of Gram negative isolates for both manual read and automatic read was within ±1 dilution of the mode for all antimicrobials tested for 28% of results from all study sites. Where the mode could not be calculated, the median was used.

Clinical isolate testing
Essential and categorical agreements (EA and CA)
As shown in Table 1, of the 390 clinical isolates tested during the study, EA was >91%, and the CA was >95%, for all cephalosporins, for all organism groups tested, for both manual read and automatic read methods.

TABLE 1. Percentage essential agreement (EA) and categorical agreement (CA) for manual and automatic reads

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Read type</th>
<th>EA (%)</th>
<th>CA (%)</th>
</tr>
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<tr>
<td>Cephalaxin</td>
<td>Manual</td>
<td>99.5</td>
<td>99.3</td>
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<tr>
<td></td>
<td>Auto</td>
<td>99.3</td>
<td>98.9</td>
</tr>
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<td>Cefuroxime</td>
<td>Manual</td>
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<tr>
<td></td>
<td>Auto</td>
<td>95.5</td>
<td>98.6</td>
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<td>Cefixime</td>
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<td>96.0</td>
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<tr>
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</table>

CONCLUSIONS
The performance of the cephalosporin antimicrobials tested on the Sensititre susceptibility system met the reproducibility, essential and categorical agreement acceptance criteria as stipulated in ISO 20776-2. Overall, the Sensititre susceptibility system proved to be an accurate alternative to standard broth microdilution methods MIC determination of cephalaxin, cefuroxime, cefixime, cefpodoxime, cefotaxime and cefepime for Gram negative organisms using EUCAST breakpoints.

REFERENCES
3. European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints v6.0

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