

# A Multi-Site Study of Tigecycline MIC Results on the Sensititre® Automated/Manual Susceptibility Plate to the NCCLS Broth Microdilution Method

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## ABSTRACT

**Background:** A multi-site comparison was performed to evaluate Tigecycline (TIG) (Wyeth-Ayerst Pharmaceuticals, Philadelphia, PA) MIC results on the Sensititre susceptibility dried plate (TREK Diagnostic Systems, Cleveland, OH). Both automated and manual reading methodologies were tested against the NCCLS reference frozen broth microdilution method (BMD).

**Methods:** TIG (0.008-16 µg/ml) was tested against 299 fresh clinical gram-positive isolates, 305 recent clinical gram-negative isolates, 75 CDC gram-positive challenge isolates and 75 CDC gram-negative challenge isolates. Sensititre plates were inoculated and read per the manufactures instructions. BMD was performed in Mueller-Hinton broth (NCCLS M7-A6). The recommended NCCLS quality control organisms were tested daily and were within the NCCLS expected TIG quality control range.

**Results:** Comparisons of TIG MIC results from the BMD method to both autoread and manually read Sensititre dried susceptibility plate were performed. Percent essential agreement (EA) rates within ± one log<sub>2</sub> dilution were determined. The EA for the gram-positive isolates on the autoread method was 97.7% and 93.3% for the challenge isolates. The EA for the gram-negative isolates using the manual read method was 98.5% and 97.4% for the challenge isolates.

**Conclusions:** This multi-site comparison of TIG on the Sensititre susceptibility system for both gram-positive and gram-negative organisms gave reliable results using either the automated or manual read methodology compared to the reference BMD method.

## PURPOSE OF THE STUDY

Tigecycline, a glycylcycline, is a new broad-spectrum, protein-inhibiting antibacterial compound.<sup>3</sup> The purpose of the study was to evaluate the performance of Tigecycline on the Sensititre® 18 – 24 hour susceptibility plate compared to the NCCLS (CLSI) reference broth microdilution method (M7 – A6).

## MATERIALS & METHODS

Antimicrobial Tested	Range Tested	Supplied By
Tigecycline (TIG)	0.008 – 16 µg/ml	Wyeth-Ayerst Pharm. (Philadelphia, PA)

### Organisms: The testing at 3 sites consisted of the following:

- 605 fresh clinical isolates (combined sites) (306 gram negative and 299 gram positive isolates)

- 150 CDC challenge isolates tested at one site (75 gram positive and 75 gram negative)

- 50 inter-laboratory reproducibility isolates tested at all 3 sites (25 gram positive and 25 gram negative)

- 3 Quality Control strains (20 times each per site):  
*E. coli* ATCC 25922  
*S. aureus* ATCC 29213  
*E. faecalis* ATCC 29212

### Susceptibility Testing Methods:

- Each isolate was tested using a Sensititre 18 – 24 h susceptibility plate containing Tigecycline. The plates were set-up and tested according to the manufacturers' instructions.
- The reference plate with Tigecycline was prepared according to the broth microdilution methods published by the Clinical Laboratory Standards Institute (CLSI, M7-A6). (The Mueller Hinton Broth used for preparing the Tigecycline reference plate was less than 12 hrs. old)
- Inter-laboratory reproducibility testing consisted of 25 gram positive and 25 gram negative isolates. The results from each laboratory were compared to the reference method results and the test plate results.

## MATERIALS & METHODS cont.

### Clinical Isolates tested

Gram Positive Organisms	Number Tested
<i>Staphylococcus aureus</i>	92
<i>Coagulase Negative Staph. spp.</i>	85
<i>Enterococcus faecalis</i>	63
<i>Enterococcus faecium</i>	30
<i>Streptococcus species Group B</i>	24
<i>Streptococcus species Group A</i>	5
<b>Total</b>	<b>299</b>

Gram Negative Organisms	Number Tested
<i>Escherichia coli</i>	46
<i>Klebsiella spp.</i>	48
<i>Enterobacter spp.</i>	32
<i>Providentia spp.</i>	12
<i>Proteus spp.</i>	42
<i>Citrobacter spp.</i>	34
<i>Morganella morganii</i>	12
<i>Serratia spp.</i>	17
<i>Acinetobacter spp.</i>	17
<i>Pseudomonas aeruginosa</i>	35
<i>Pseudomonas spp.</i>	11
<b>Total</b>	<b>306</b>

## RESULTS

### Quality Control Testing

Quality Control Organism Tested	QC Range (µg/ml) and Frequency of Results					
<i>S. aureus</i> ATCC 29213	0.015	0.03	0.06	0.12	0.25	0.5
	Autoread					33 26 1
	Manual read					56 4
<i>E. faecalis</i> ATCC 29212	0.015	0.03	0.06	0.12	0.25	0.5
	Autoread					50 9 1
	Manual read					50 9 1
<i>E. coli</i> ATCC 25922	0.015	0.03	0.06	0.12	0.25	0.5
	Autoread					60
	Manual read					57 3

## RESULTS cont.

### Clinical Isolates

#### Manual Read Method

Organisms	Total Isolates Tested	Isolates in Agreement	% Essential Agreement (+/- one log <sub>2</sub> dilution)
<i>Enterobacteriaceae</i>	243	240	98.7
<i>Non- Enterobacteriaceae</i>	63	59	93.6
<i>Staphylococcus spp.</i>	177	177	100
<i>Enterococcus spp.</i>	93	92	99
<i>Group A &amp; B Streptococcus spp.</i>	29	27	93
<b>Totals</b>	<b>605</b>	<b>595</b>	<b>98.3</b>

#### Auto Read Method

Organisms	Total Isolates Tested	Isolates in Agreement	% Essential Agreement (+/- one log <sub>2</sub> dilution)
<i>Enterobacteriaceae</i>	243	241	99
<i>Non- Enterobacteriaceae</i>	63	61	96.8
<i>Staphylococcus spp.</i>	177	175	98.8
<i>Enterococcus spp.</i>	93	90	96.7
<i>Group A &amp; B Streptococcus spp.</i>	29	27	93
<b>Totals</b>	<b>605</b>	<b>594</b>	<b>98.2</b>

## Challenge Isolates

#### Manual Read Method

Organisms	Total Isolates Tested	Isolates in Agreement	% Essential Agreement (+/- one log <sub>2</sub> dilution)
Gram Negative	75	71	94.7
Gram Positive	75	71	94.7
<b>Totals</b>	<b>150</b>	<b>142</b>	<b>94.7</b>

#### Auto Read Method

Organisms	Total Isolates Tested	Isolates in Agreement	% Essential Agreement (+/- one log <sub>2</sub> dilution)
Gram Negative	75	71	94.7
Gram Positive	75	70	93.3
<b>Totals</b>	<b>150</b>	<b>141</b>	<b>94</b>

## RESULTS cont.

### Gram Negative Inter-laboratory Reproducibility Isolates vs. Tigecycline Essential Agreement Within +/- one log<sub>2</sub> Dilution of Reference MIC

Site	# Agreement Manual	#Agreement Autoread	Total Isolates Tested	Manual%	Autoread%
1	25	25	25	100	100
2	25	25	25	100	100
3	25	25	25	100	100
<b>Combined</b>	<b>75</b>	<b>75</b>	<b>75</b>	<b>100</b>	<b>100</b>

### Gram Positive Inter-laboratory Reproducibility Isolates vs. Tigecycline Essential Agreement Within +/- one log<sub>2</sub> Dilution of Reference MIC

Site	# Agreement Manual	#Agreement Autoread	Total Isolates Tested	Manual%	Autoread%
1	25	25	25	100	100
2	25	24	25	100	96
3	25	25	25	100	100
<b>Combined</b>	<b>75</b>	<b>74</b>	<b>75</b>	<b>100</b>	<b>98.6</b>

## CONCLUSIONS

This study compared the Sensititre® 18 – 24 hour susceptibility plate with the CLSI reference microdilution frozen plate (M7–A6). The Sensititre plate performed equivalent to the standard reference microbroth dilution method.

### Clinical Isolates:

The overall essential agreement for Tigecycline, within +/- one log<sub>2</sub> dilution, was 98.3% for the manual method and 98.2% for the autoread method.

### CDC Challenge Organisms:

The overall essential agreement for Tigecycline, within +/- one log<sub>2</sub> dilution, was 94.6% for the manual method and 94% for the autoread method.

### Inter-laboratory Reproducibility:

All Inter-laboratory reproducibility testing results, both autoread and manual read, were within +/- one log<sub>2</sub> dilution of expected result.

Inter-laboratory reproducibility testing essential agreement, within +/- one log<sub>2</sub> dilution of the reference method result, was 100% for the manual read method and 99.3% for the autoread method.

## REFERENCES

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