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A Multi-Site Study of an 18-24h Susceptibility System to the CLSI Microdilution Method for Gram Negative and Gram Positive Organisms vs. Doxycycline

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ABSTRACT

Background: Doxycycline (DOX) (Pfizer Pharmaceuticals, Groton, CT), a synthetic antibiotic derived from tetracycline, is effective in treating a variety of bacterial infections. An evaluation was performed to determine the accuracy and reproducibility of the Sensititre® dried susceptibility system (TREK Diagnostic Systems, Cleveland, OH) using both automated and manual reading methodologies with DOX compared to the CLSI M7 reference broth microdilution method (BMD).
Materials and Methods: DOX (0.03 - 16 µg/ml) was tested against 905 fresh clinical isolates, 132 challenge isolates (130 Coag-neg *Staphylococcus*, 178 *Staphylococcus aureus*, 121 *Enterococcus* spp., 99 *Streptococcus* spp., 88 *Acinetobacter* spp., 118 Non-enterobacteriaciae and 315 Enterobacteriaciae) and 50 reproducibility isolates. Dried plates were inoculated as per manufacturers' instructions, and the BMD was performed as per CLSI M7. Recommended CLSI quality control (QC) organisms were tested daily and were within the CLSI expected QC ranges.
Results: Comparisons for DOX MIC results on the Sensititre system to the CLSI M7 BMD for both automated and manual reads, resulted in 97.9/99.5% essential agreement for DOX (+/- one log_2 dilution). Regarding categorical agreements, the results did not show very major or major errors and had a ≤10% minor error rate for all isolates. Reproducibility was calculated as the percentage of results within +/- one log_2 dilution of the modal MIC. Overall agreement for the reproducibility, autoread and manually read, for DOX was 98.5/98% respectively.
Conclusions: The results for DOX indicates that the Sensititre susceptibility system for all clinical and challenge isolates gave reliable results using either the automated/manual read method compared to the reference BMD.
***Essential agreement rates are subject to change**

INTRODUCTION

Doxycycline, is a semi-synthetic tetracycline developed in the early 1960s by Pfizer Inc. Doxycycline was Pfizer's first once-a-day broad spectrum antibiotic and is commonly used in treating a variety of bacterial infections.

PURPOSE OF THE STUDY

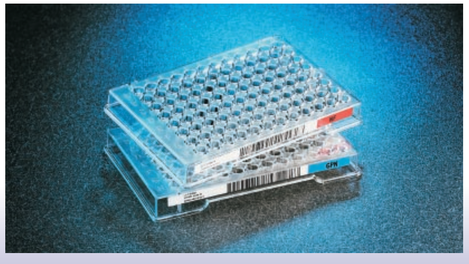
A multi-site study to evaluate the performance of Doxycycline on the Sensititre® 18 – 24 hour automated/manual susceptibility plate compared to the CLSI Microdilution Method System.

Antimicrobials Tested

Antimicrobials Tested	Range Tested µg/ml	Supplied By
Doxycycline (DOX)	0.03 – 16µg/ml	Pfizer Pharmaceuticals, Groton, CT

Organisms Tested

Clinical Isolates (combined sites)	905
CDC challenge isolates (one site)	132
Reproducibility isolates (combined sites)	50
CLSI Quality Control Strains (combined sites)	180



MATERIALS & METHODS cont.

Clinical and Challenge Isolates tested

Gram Positive Organisms	Number Tested
Staphylococcus aureus	178
Coagulase Negative Staph. spp.	130
Enterococcus faecalis	51
Enterococcus faecium	63
Enterococcus spp.	7
Streptococcus species Group B	49
Streptococcus species Group A	40
Total	**518**

Gram Negative Organisms	Number Tested
Escherichia coli	81
Klebsiella spp.	60
Enterobacter spp.	42
Providencia spp.	16
Proteus spp.	41
Citrobacter spp.	32
Morganella morganii	17
Serratia spp.	24
Acinetobacter baumannii	60
Acinetobacter spp.	28
Pseudomonas aeruginosa	68
Pseudomonas spp.	50
Total	**519**

Quality Control

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

Quality Control Strains	CLSI MIC Ranges (µg/ml)
Staphylococcus aureus ATCC 29213	0.12-0.5
Enterococcus faecalis ATCC 29212	2-8
Escherichia coli ATCC 25922	0.5-2

SUSCEPTIBILITY TESTING METHODS

- Indications for use: The Sensititre 18-24 hour MIC or breakpoint susceptibility system is an *in vitro* diagnostic product for clinical susceptibility testing of non-fastidious organisms.
- Each isolate was tested using a Sensititre 18 – 24 susceptibility plate containing **Doxycycline**. The plates were set-up and tested according to the manufacturers' instructions.
- The CLSI reference microdilution plate was prepared and tested according to the Clinical Laboratory Standards Institute (CLSI M7).
- Testing consisted of 905 fresh clinical isolates; approximately 300 isolates, both Gram positive and Gram negative, supplied by each site and 132 challenge isolates supplied to a single testing site.
- Reproducibility testing consisted of 25 Gram positive and 25 Gram negative tested at all 3 sites on the Sensititre 18-24 hour susceptibility plate. The test plate results were compared to the CLSI reference microdilution results.
- Quality control consisted of testing 20 replicates of each ATCC strain, including *S. aureus* 29213, *E. faecalis* 29212 and *E.coli* 25922, at each site.
- Colony counts were performed on the QC on each day of testing.

RESULTS

Essential agreement for the clinical and challenge isolates were calculated using the +/- one log_2 dilution standard for comparison studies. The categorical agreement was calculated using FDA interpretations. The essential agreement rates for the clinical and challenge isolates were as follows:

Overall Essential and Categorical Agreements of Gram Positive Clinical and Challenge Isolates using the Auto Read Methodology

Clinical Isolates (Test Results × Modal MIC)

Test Results \ Modal MIC	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
0.03	1	1									
0.06	1	24	41	1							
0.12		32	131	21							
0.25		2	4	2	4						
0.5					22	10					
1					5	14	8				
2							3	4			
4							1	14	5		
8								2	65		
16									4	18	4
>16											3
TOTALS	2	59	176	24	31	24	12	20	74	18	7

Challenge Isolates (Test Results × Modal MIC)

Test Results \ Modal MIC	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
0.03	1	1	1	1							
0.06		2	9								
0.12			19	7							
0.25			2								
0.5						4					
1											
2							1	2			
4								1	1		
8									2	8	1
16										1	3	2
>16											1	1
TOTALS	1	3	31	8	0	4	1	5	10	5	3

FDA Interpretive Criteria — Sensitive <=4 | Intermediate -8 | Resistant >=16
Essential Agreement 98.2%

Overall Essential and Categorical Agreements of Gram Negative Clinical and Challenge Isolates using the Auto Read Methodology

Clinical Isolates (Test Results × Modal MIC)

Test Results \ Modal MIC	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
0.03	1	1									
0.06	2	7	4								
0.12	1	1	6	2							
0.25			3	4	1						
0.5					4	16					
1					2	34	31	2			
2						6	62	27			
4							2	20	8		
8								1	37	17	1
16									2	26	26
>16									1	1	99
TOTALS	4	9	13	6	7	56	95	50	48	44	126

Challenge Isolates (Test Results × Modal MIC)

Test Results \ Modal MIC	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
0.03	1										
0.06											
0.12			1								
0.25				1							
0.5					1	3					
1						6	3				
2							8	2			
4							1	2	1		
8									8	1	
16										3	4
>16											20
TOTALS	1	0	1	1	1	9	12	4	9	4	24

FDA Interpretive Criteria — Sensitive <=4 | Intermediate -8 | Resistant >=16
Essential Agreement 99.4%

Overall Essential and Categorical Agreements of Gram Positive Clinical and Challenge Isolates using the Manual Read Methodology

Clinical Isolates (Test Results × Modal MIC)

Test Results \ Modal MIC	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
0.03	1	1									
0.06	1	35	24								
0.12		23	137	18							
0.25			15	6	1						
0.5					25	8					
1					5	17	6				
2							6	1			
4								14	3		
8								2	68	2	
16									3	17	4
>16										2	3
TOTALS	2	59	176	24	31	25	12	17	74	21	7

Challenge Isolates (Test Results × Modal MIC)

Test Results \ Modal MIC	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
0.03	1	1									
0.06		2	7								
0.12			22	6							
0.25			2	1							
0.5						1					
1						3					
2							1				
4								4	1		
8								1	9	1	
16										4	1
>16											3
TOTALS	1	3	31	7	0	4	1	5	10	5	4

FDA Interpretive Criteria — Sensitive <=4 | Intermediate -8 | Resistant >=16
Essential Agreement 100%

Overall Essential and Categorical Agreements of Gram Negative Clinical and Challenge Isolates using the Manual Read MethodologyModal MIC

Clinical Isolates (Test Results × Modal MIC)

Test Results \ Modal MIC	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
0.03	1	1									
0.06	3	6	3								
0.12		2	9	2							
0.25			1	4	2						
0.5					3	13					
1					2	36	22				
2						7	69	23			
4							5	23	6		
8								4	38	18	3
16									4	22	20
>16									1		105
TOTALS	4	9	13	6	7	56	96	50	49	40	128

Challenge Isolates (Test Results × Modal MIC)

Test Results \ Modal MIC	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
0.03	1										
0.06											
0.12			1								
0.25				1							
0.5						2					
1					1	7	5				
2							7	2			
4								2	1		
8									8	2	
16										2	4
>16											20
TOTALS	1	0	1	1	1	9	12	4	9	4	24

FDA Interpretive Criteria — Sensitive <=4 | Intermediate -8 | Resistant >=16
Essential Agreement 99.5%

RESULTS cont.

Interlaboratory Reproducibility % Essential and Categorical Agreements +/- one log_2 Dilution of the Modal MIC

	Auto Gram Positive	Manual Gram Positive	Auto Gram Negative	Manual Gram Negative
Between-site total isolates tested	75	75	75	75
Between-site isolates within +/- 1 well from mode	75	75	74	72
Between-site reproducibility ratio	75/75	75/75	74/75	72/75
Between-site reproducibility %	100%	100%	99%	96%
Total Essential Agreement	75	75	74	74
Essential Agreement %	100%	100%	99%	99%
Total Categorical Agreement	75	75	75	75
Categorical Agreement	70	71	74	72
Categorical Agreement %	93%	95%	99%	96%

CONCLUSION

The Sensititre 18 – 24 hour susceptibility system when compared to the CLSI M7 reference microdilution plate demonstrated an equivalent level of performance when testing **Doxycycline** vs. Gram positive and Gram negative clinical and challenge isolates.

- **Clinical Isolates and CDC Challenge Organisms**
  The overall essential agreement for **Doxycycline** and Gram positive isolates, within +/- one log_2 dilution, was 100% for the manual method and 98.1% for the autoread method.

  The overall essential agreement for **Doxycycline** and Gram negative isolates, within +/- one log_2 dilution, was 99.5% for the manual method and 99.4% for the autoread method.

  The overall categorical agreements for **Doxycycline** did not show very major or major errors and demonstrated a ≤10% minor error rate for all isolates with categorical interpretations.

- **Interlaboratory Reproducibility:**
  Reproducibility testing results, within +/- one log_2 dilution from the expected result, were 99.5% for the autoread method and 98% for the manual read method.

REFERENCES

1. Clinical and Laboratory Standards Institute. 2006. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-sixth edition.* Approved document M7-A7. Wayne, PA: CLSI.

2. Clinical and Laboratory Standards Institute. 2008. *Performance standards for antimicrobial susceptibility testing, 18th informational supplement* M100-S18. Wayne, PA: CLSI.