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A Multi-Site Comparison Study of the Sensititre® Automated/Manual Susceptibility Plate to the CLSI Microdilution Method for *Streptococcus* spp.

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ABSTRACT

Background: A multi-site study was performed to evaluate performance of the Sensititre susceptibility plate (TREK Diagnostic Systems, Cleveland, OH) for *Streptococcus spp.*, including viridans *Streptococcus* group and beta hemolytic Streptococcus, against 18 commonly used antimicrobials. Both automated and manual reading methodologies were tested against the CLSI-M7 reference broth microdilution method (BMD).
Methods: The antimicrobials were tested against 299 fresh clinical, 50 CDC challenge, and 25 reproducibility isolates consisting of viridans *Streptococcus* and beta hemolytic *Streptococcus* group A, B, C, F, and G. Sensititre plates were inoculated and incubated at 35°C for 24 hours, and read as per the manufacturer's instructions. BMD was performed in Mueller-Hinton broth supplemented with 2-5% lysed horse blood as per CLSI M7. The recommended CLSI quality control organism *Streptococcus pneumoniae* ATCC 49619 was tested daily and performed within the specified ranges for each antimicrobial. A second internal quality control organism of *Streptococcus pneumoniae* was also tested daily and performed within previously determined ranges.
Results: The CLSI reference BMD plates were compared to autoread and manually read Sensititre susceptibility plates. Percent essential agreement (EA) rates within +/- one log_2 dilution were determined. EA for the autoread method with the clinical and challenge isolates was 99.7% and 100% respectively. EA for the manual read method with the clinical and challenge isolates was 99.9% and 100% respectively. Categorical Agreement with the autoread and manual read methods was 98.9% and 99.2 % respectively. EA for the 25 reproducibility isolates using the automated and manual methods was at 99.6% and 99.6% respectively.
Conclusions: This multi-site comparison of the Sensititre susceptibility system for *Streptococcus spp.* gave reliable results using the automated and manual read methodology when compared to the reference BMD.

PURPOSE OF THIS STUDY

To perform a multi-site study to evaluate the performance of the Sensititre AutoReader using Sensititre dried 20-24 hour susceptibility plates for testing *Streptococcus spp.*, including viridans *Streptococcus* group and beta hemolytic *Streptococcus* against 18 commonly used antimicrobials compared to the CLSI M7 reference broth microdilution method.

MATERIALS & METHODS

Organisms

The testing at 3 sites consisted of the following:

- 299 Clinical isolates
- 50 CDC Challenge isolates
- 25 Reproducibility isolates
- Quality Control strains

  Streptococcus pneumoniae ATCC 49619 and in house *Streptococcus pneumoniae* isolate #5.

Isolates

Clinical and Challenge Isolates Tested	
viridans *Streptococcus* group	127
beta hemolytic group A *Streptococcus*	90
beta hemolytic group B *Streptococcus*	88
beta hemolytic group C *Streptococcus*	17
beta hemolytic group G *Streptococcus*	19
beta hemolytic group F *Streptococcus*	8
Total	**349**

MATERIALS & METHODS

Antimicrobials

Antimicrobials Tested	Range Tested (µg/ml)	Supplied By
Amoxicillin/clavulanic acid	2/1-16/8	Sigma/GSK
Azithromycin	0.25-2	Pfizer
Cefepime	0.12 – 8	BMS
Cefotaxime	0.12 - 4	Sigma
Ceftriaxone	0.06 – 2	Sigma
Cefuroxime	0.5 - 4	Sigma
Chloramphenicol	2-16	Sigma
Erythromycin	0.25 - 2	Sigma
Gatifloxacin	0.5 - 8	BMS
Gemifloxacin	0.03 – 0.5	LG Life Sciences
Levofloxacin	0.5 – 16	J&J
Linezolid	0.25-4	Pfizer
Meropenem	0.25 – 2	Astra Zenica
Moxifloxacin	0.25 – 8	Bayer Health
Penicillin	0.03 – 8	Sigma
Tetracycline	0.5 – 8	Sigma
Trimethoprim/sulfamethoxazole	0.5/9.5 - 4/76	Sigma
Vancomycin	0.5 - 4	Amresco

SUSCEPTIBILITY TESTING METHODS

- Each *Streptococcus spp.* including viridans *Streptococcus* group and beta hemolytic *Streptococcus*, was tested using a Sensititre dried susceptibility plate. The plates were set up and tested according to the manufacturer's instructions.
- Reference plates were tested according to the BMD methods published in the Clinical and Laboratory Standards Institute (CLSI, M7-A7).

Quality Control Testing

Antimicrobials Tested	ATCC 49619 (µg/ml)	#5 In House QC (µg/ml)
Amoxicillin/clavulanic acid	0.03/0.015-0.12/0.06	1/0.5-4/2
Azithromycin	0.06-0.25	1-4
Cefepime	0.03-0.25	0.5-2
Cefotaxime	0.03-0.12	0.5-2
Ceftriaxone	0.03-0.12	1-4
Cefuroxime	0.25-1	4-16
Chloramphenicol	2-8	>4
Erythromycin	0.03-0.12	>0.5
Gatifloxacin	0.12-0.5	0.25-1
Gemifloxacin	0.008-0.03	0.015-0.06
Levofloxacin	0.5-2	0.5-2
Linezolid	0.5-2	0.5-2
Meropenem	0.06-0.25	0.5-2
Moxifloxacin	0.06-0.25	0.06-0.25
Penicillin	0.25-1	>1
Tetracycline	0.12-0.5	>4
Trimethoprim/sulfamethoxazole	0.12/2.4-1/19	>2/38
Vancomycin	0.12-0.5	0.25-1

RESULTS

Table 1. Interlaboratory Reproducibility % Essential Agreement +/- 1 log_2 Dilution of the Modal MIC

Antimicrobial	Autoreader	Manual
Amoxicillin/clavulanic acid	100	100
Azithromycin	97	97
Cefepime	100	100
Cefotaxime	100	100
Ceftriaxone	99	97
Cefuroxime	100	100
Chloramphenicol	100	100
Erythromycin	99	99
Gatifloxacin	100	100
Gemifloxacin	99	99
Levofloxacin	100	100
Linezolid	99	97
Meropenem	100	100
Moxifloxacin	100	100
Penicillin	100	100
Tetracycline	100	100
Trimethoprim/sulfamethoxazole	100	96
Vancomycin	100	100
Total	**99.6**	**99.2**

Table 2. Overall % Essential and Categorical Agreements of Clinical and Challenge Isolates using the Manual Read Methodology

Antimicrobial	% Essential Agreement	% Categorical Agreement	Minor Errors	Major Errors	Very Major Errors
Amoxicillin/clavulanic acid	100	-	-	-	-
Azithromycin	100	98.8	5	0	0
Cefepime	99.4	98.3	4	0	0
Cefotaxime	100	98.9	4	0	0
Ceftriaxone	99.4	98.6	5	0	0
Cefuroxime	100	-	-	-	-
Chloramphenicol	100	99.4	2	0	0
Erythromycin	100	98.9	4	0	0
Gatifloxacin	100	99.4	2	0	0
Gemifloxacin	100	-	-	-	-
Levofloxacin	100	100	0	0	0
Linezolid	100	100	0	0	0
Meropenem	100	100	0	0	0
Moxifloxacin	100	-	-	-	-
Penicillin	99.7	99.1	3	0	0
Tetracycline	100	98.6	5	0	0
Trimethoprim/sulfamethoxazole	99.7	-	-	-	-
Vancomycin	100	100	0	0	0
Total	**99.9**	**99.2**	**34**	**0**	**0**

RESULTS con't

Table 3. Overall % Essential and Categorical Agreements of Clinical and Challenge Isolates using the Autoread Methodology

Antimicrobial	% Essential Agreement	% Categorical Agreement	Minor Errors	Major Errors	Very Major Errors
Amoxicillin/clavulanic acid	100	-	-	-	-
Azithromycin	99.7	97.7	8	0	0
Cefepime	99.4	98.6	2	0	0
Cefotaxime	100	98	7	0	0
Ceftriaxone	98.6	98.6	3	0	0
Cefuroxime	100	-	-	-	-
Chloramphenicol	99.4	98.6	4	1	0
Erythromycin	100	98.6	5	0	0
Gatifloxacin	100	100	0	0	0
Gemifloxacin	100	-	-	-	-
Levofloxacin	100	100	0	0	0
Linezolid	100	100	0	0	0
Meropenem	100	100	0	0	0
Moxifloxacin	100	-	-	-	-
Penicillin	98.6	97.1	7	0	0
Tetracycline	99.7	98.9	3	1	0
Trimethoprim/sulfamethoxazole	99.7	-	-	-	-
Vancomycin	100	100	0	0	0
Total	**99.7**	**98.9**	**39**	**2**	**0**

CONCLUSION

This study compared the Sensititre dried susceptibility plate with the CLSI M7 reference BMD method. The Sensititre dried susceptibility plate read by the Autoreader and manually read, performed equivalent to the standard reference BMD method.

Clinical and Challenge Isolates:

- The overall essential agreement for 349 isolates within a +/- one log_2 dilution range, was 99.9% for the manual method and 99.7% the for autoread method.
- The overall categorical agreement for 349 isolates was 99.2% for the manual method and 98.9% for the autoread method.

Reproducibility:

- Interlaboratory reproducibility essential agreement was 99.2 % for the manual read method and 99.6 % for the autoread method.

REFERENCES

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2. Clinical and Laboratory Standards Institute. 2006. *Performance standards for antimicrobial susceptibility testing, 16th informational supplement* M100-S16. Wayne, PA: CLSI.