Paper # **A-010**

A Multi-Site Study of a Broth Cefoxitin Screen Test Using TREK Sensititre® 18-24 hour dried Susceptibility Plates for Predicting the Presence of *mecA*-mediated Resistance in *Staphylococcus aureus*

C. C. Knapp[1] , C. M. Bastulli[1], N. M. Holliday[1], S. B. Killian[1], A. Miskov[1], J. Streit[2], D. Biedenbach[2], R. Jones[2], J. Beach[3], L. Define[3], J. DiPersio[3]

1. TREK Diagnostic Systems, Cleveland, OH 2. JMI Laboratories, North Liberty, IA 3. Summa Health System, Akron, OH

TREK DIAGNOSTIC SYSTEMS
MAGELLAN BIOSCIENCES

982 Keynote Circle, Suite 6
Cleveland, OH 44131
800.871.8909 • www.trekds.com

ABSTRACT

Background: A multi-site study was performed to evaluate a broth microdilution cefoxitin screen test on the Sensititre 18-24 hour dried susceptibility plates (TREK Diagnostic Systems, Cleveland, Ohio) for predicting the presence of *mecA*-mediated resistance in *Staphylococcus aureus.* The Sensititre dried plates were both auto and manually read and were compared to the CLSI M100 reference method results.
Methods: The cefoxitin screen test on the Sensititre dried plate was tested against 207 clinical and challenge strains of *Staphylococcus aureus.* The recommended CLSI quality control organisms, *Staphylococcus aureus* ATCC 29213 and ATCC 25923 were tested daily. Sensititre plates were set up and read as per the manufacturer's instructions. The reference cefoxitin screen was performed following the CLSI M100 reference method.
Results: The cefoxitin screen test was autoread and manually read, and compared to the CLSI reference method. The sensitivity and specificity for *Staphylococcus aureus* autoread were 100% and 99.3% respectively. The sensitivity and specificity for *Staphylococcus aureus* read manually were 100% and 99.6% respectively.
Conclusions: The assessment of the broth cefoxitin screen test on the Sensititre dried plates to predict the presence of *mecA*-mediated resistance in *Staphylococcus aureus*, provided reliable results using both the auto and manual read methodology compared to the CLSI reference method.

INTRODUCTION

The cefoxitin screen disk diffusion test and the BMD test as per CLSI M100 are both recommended for detection of the *mecA* gene in *Staphylococcus aureus.* The purpose of this study was to investigate the possibility of using the Sensititre 18-24 hour susceptibility system to determine the presence of the *mecA* gene in *Staphylococcus aureus*, giving labs the ability to meet all of their testing needs on one platform. All Sensititre Susceptibility results were determined by both automated reading of enzymatic release of fluorophore using the Sensititre AutoReader and by manual visual method.

MATERIALS & METHODS

Antimicrobial Tested

Antimicrobials Tested	Concentration
Cefoxitin	6 µg/mL
Supplier Disk Supplier	Sigma-Aldrich St. Louis, Missouri Becton, Dickinson and Company Franklin Lakes, NJ

Number of Isolates Tested

Organism	Clinical	Challenge	CDC Challenge	Reproducibility
Staphylococcus aureus	150	27	30	19

Quality Control Isolates

Organism	ATCC Number
Staphylococcus aureus	25923
Staphylococcus aureus	29213
Staphylococcus aureus	BAA-976

SUSCEPTIBILITY TESTING METHODS

- Each isolate was tested using a Sensititre 18-24 hour susceptibility plate containing cefoxitin screen test (6 µg/ml) for detection of the *mec*-A gene in *Staphylococcus aureus.* The plates were set-up and tested according to the manufacturers' instructions.
- The CLSI reference cefoxitin disk diffusion test and BMD tests were performed on each isolate according to Clinical Laboratory Standards Institute M2 and M100.
- Testing consisted of 150 fresh clinical *S.aurues* isolates, performed at 3 sites. 27 *S.aureus* challenge strains and 30 *S.aureus* CDC challenge strains tested at a single site. 19 Reproducibility strains were tested at each site.
- QC and QA isolates were tested at each test site on each test day and consisted of 20 replicates of ATCC strains, *Staphylococcus aureus* 29213, *Staphylococcus aureus* BAA-976, *Staphylococcus aureus* 25923.

RESULTS

Sensitivity and Specificity between the reference disk diffusion test and the cefoxitin broth screen test were determined for *Staphylococcus aureus.*

Cefoxitin Screen Test
Clinical Autoreader Read

Sensititre Autoread		Reference BMD/FOX DISK		TOTAL
		Susceptible	Resistant	
TEST	Susceptible	58/60	2/1	60/61
	Resistant	0/0	90/89	90/89
TOTAL		58/60	92/90	150/150

Cefoxitin Screen Test
Clinical Manual Read (SWIN)

Sensititre Manual		Reference BMD/FOX DISK		TOTAL
		Susceptible	Resistant	
TEST	Susceptible	59/60	1/0	60/60
	Resistant	0/0	90/90	90/90
TOTAL		59/60	91/90	150/150

RESULTS cont.

Cefoxitin Screen Test
Challenge Autoreader Read (SWIN)

Sensititre Autoread		Reference BMD/FOX DISK		TOTAL
		Susceptible	Resistant	
TEST	Susceptible	16/16	0/0	16/16
	Resistant	0/0	11/11	11/11
TOTAL		16/16	11/11	27/27

Cefoxitin Screen Test
Challenge Manual Read (SWIN)

Sensititre Manual		Reference BMD/FOX DISK		TOTAL
		Susceptible	Resistant	
TEST	Susceptible	16/16	0/0	16/16
	Resistant	0/0	11/11	11/11
TOTAL		16/16	11/11	27/27

Cefoxitin Screen Test
CDC Challenge Autoreader Read (SWIN)

Sensititre Autoread		CDC Reference Test Plate/FOX DISK		TOTAL
		Susceptible	Resistant	
TEST	Susceptible	13/13	0/0	13/13
	Resistant	0/0	17/17	17/17
TOTAL		13/13	17/17	30/30

Cefoxitin Screen Test
CDC Challenge Manual Read (SWIN)

Sensititre Manual		CDC Reference Test Plate/FOX DISK		TOTAL
		Susceptible	Resistant	
TEST	Susceptible	13/13	0/0	13/13
	Resistant	0/0	17/17	17/17
TOTAL		13/13	17/17	30/30

RESULTS cont.

Reproducibility Results for *Staphylococcus aureus*

	Total	Cefoxitin				% Essential Agreement with Broth Cefoxitin Screen test Method
		Test Positive	Reference Positive	Test Negative	Reference Negative	
AUTO	57	37	39	20	18	96.5%
MANUAL	57	37	39	20	18	96.5%

CONCLUSION

The cefoxitin screen test (6µg/mL) on the Sensititre 18-24 hour susceptibility system when compared to the CLSI reference disk diffusion and BMD methods, demonstrated an equivalent level of performance when testing for detection of the *mecA* gene in *Staphylococcus aureus.*

% Sensitivity and Specificity for the Cefoxitin Screen Test v Disk Diffusion

Disk Diffusion vs.	Clinical	Challenge	CDC Challenge
Sensitivity Autoread	100	100	100
Specificity Autoread	100	100	100
Sensitivity Manual	100	100	100
Specificity Manual	99	100	100

% Sensitivity and Specificity for the Cefoxitin Screen Test v BMD

BMD vs.	Clinical	Challenge	CDC Challenge
Sensitivity Autoread	100	100	100
Specificity Autoread	99	100	100
Sensitivity Manual	100	100	100
Specificity Manual	98	100	100

The high level of agreement between the Sensititre 18-24 hour susceptibility systems cefoxitin screen test and the CLSI reference methodologies suggest the potential value of this method for clinical laboratories to eliminate offline testing.

REFERENCES

1. Clinical and Laboratory Standards Institute. 2006. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Ninth Edition Approved document M2-A9

2. Clinical and Laboratory Standards Institute. 2008. Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Information Supplement M100-S18.