Paper # **C-013**

A Multi-Site Study of a Broth D Zone Test System using TREK Sensititre® 18-24 Hour Dried Susceptibility Plates for Detecting Inducible Resistance to Clindamycin in Macrolide Resistant Gram-Positive Organisms

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ABSTRACT

Background: A multi-site study was performed to evaluate a broth D zone test on the Sensititre dried susceptibility plate (TREK Diagnostic Systems, Cleveland, Ohio) for determining inducible resistance to Clindamycin in macrolide resistant gram-positive (GP) organisms. The Sensititre dried MIC plates were read manually (autoread algorithms are presently under development) and compared to the CLSI M2-A9 reference D zone agar disk approximation test.
Methods: The broth D zone MIC well is comprised of a combination of Erythromycin (Ery) and Clindamycin (Cli) which was tested against 224 fresh clinical GP isolates, 55 CDC GP Challenge strains, and 19 reproducibility strains. The recommended CLSI quality control organisms were tested daily and were in control. Sensititre plates were inoculated, incubated at 35°C for 24 hours, and read as per the manufacturer's instructions. The reference D zone test was performed in parallel and according to CLSI M2-A9.
Results: The broth D zone dried susceptibility plates read manually were compared to the reference disk D zone method. Agreement rates were determined for *Staphylococcus* species and beta hemolytic *Streptococcus* species. The Sensititre susceptibility plates read manually agreed 100% with the reference disk D zone results for both clinical, challenge, and reproducibility strains.
Conclusions: The assessment of the broth D zone test on the Sensititre dried susceptibility system to detect inducible resistance gave reliable results using manual read methodology compared to the reference D zone disk approximation test.

INTRODUCTION

A major concern in clinical laboratories today is the detection of inducible resistance to Clindamycin in macrolide resistant gram positive organisms caused by the erm gene. Clindamycin is often used by physicians for treatment of *Staphylococcus* spp. infections. The reporting of false susceptibility of *Staphylococcus* spp. to Clindamycin can have adverse effects on patient outcomes. The accurate and timely detection of these isolates are critical to patient care. The current CLSI M2 and CLSI M100 reference method for detecting erm mediated resistance is the D zone agar disk approximation test. This test can be difficult to read, and often is performed in addition to a traditional MIC plate. In the past broth microdilution (BMD) has not been able to detect erm resistance.

PURPOSE OF THIS STUDY

To evaluate the performance of a broth D zone test system using TREK Sensititre® 18-24 hour dried susceptibility plates for detecting inducible resistance to clindamycin in macrolide resistant gram-positive organisms

MATERIALS & METHODS

Table 1.1 Organisms Tested

Organism	Number of Isolates Tested	
	Clinical	Challenge
Staphylococcus aureus	98	27
Coagulase Negative *Staphylococcus*	74	17
Beta Haemolytic *Streptococcus* Group A and B	52	11
Total	**224**	**55**

MATERIALS & METHODS

Table 1.2 CLSI Quality Control Strains (40 replicates of each tested)

Organism	ATCC Number
Staphylococcus aureus	BAA-976
Staphylococcus aureus	BAA-977
Staphylococcus aureus	29213
Enterococcus faecalis	29212

Table 1.3 Antimicrobials Tested

Antimicrobials Tested	Combination Concentration
Erythromycin/Clindamycin	8/1.5 µg/ml

Antimicrobics	Supplied by
Erythromycin	Sigma Chemical
Clindamycin	Melford Laboratories

SUSCEPTIBILITY TESTING METHODS

- Each isolate was tested using a Sensititre 18-24 hour susceptibility plate containing an Erythromycin/Clindamycin well in a combination of 8/1.5 µg/ml for detection of erm mediated resistance. The plates were set-up and tested according to the manufacturer's instructions.
- The CLSI reference D zone agar approximation test was performed on each isolate according to Clinical Laboratory Standards Institute M2 and M100.
- Testing consisted of 224 fresh clinical isolates, performed at 2 sites. 55 challenge strains were supplied to a single site for testing. 19 Reproducibility strains were performed at each site.
- QC and QA isolates were tested each test day and consisted of 20 replicates performed at each site of ATCC strains *Staphylococcus aureus* BAA-977, *Staphylococcus aureus* BAA-976, *Staphylococcus aureus* 29213, and *Enterococcus faecalis* 29212.

RESULTS

Agreement rates between the reference D zone agar disk approximation test and the broth D zone BMD well were determined for both *Staphylococcus* spp. and *Streptococcus* spp. The reference D zone agar disk approximation test was read as per the CLSI M2. The BMD D zone test was read for growth or no growth as per manufacturer's instructions.

Table 1.4 Site 1 Clinical Results

Organism	Reference D zone Result		% Essential Agreement with Broth Microdilution D test Method
	Positive	Negative	
Staphylococcus aureus	14	40	100%
Coagulase Negative *Staphylococcus*	0	33	100%
Beta Haemolytic *Streptococcus* Group A and B	1	25	100%
Total	**15**	**98**	**100%**

Table 1.5 Site 2 Clinical Results

Organism	Reference D zone Result		% Essential Agreement with Broth Microdilution D test Method
	Positive	Negative	
Staphylococcus aureus	12	32	100%
Coagulase Negative *Staphylococcus*	5	36	100%
Beta Haemolytic *Streptococcus* Group A and B	0	26	100%
Total	**17**	**94**	**100%**

Table 1.6 Challenge Results

Organism	Reference D zone Result		% Essential Agreement with Broth Microdilution D test Method
	Positive	Negative	
Staphylococcus aureus	14	13	100%
Coagulase Negative *Staphylococcus*	0	17	100%
Beta Haemolytic *Streptococcus* Group A and B	0	11	100%
Total	**14**	**41**	**100%**

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Table 1.7 Site 1 Reproducibility Results

Organism	Total	Reference D zone Result		% Essential Agreement with Broth Microdilution D test Method
		Positive	Negative	
Staphylococcus aureus	9	3	6	100%
Coagulase Negative *Staphylococcus*	5	1	4	100%
Beta Haemolytic *Streptococcus* Group A and B	5	0	5	100%
Total	**19**	**4**	**15**	**100%**

Table 1.8 Site 2 Reproducibility Results

Organism	Total	Reference D zone Result		% Essential Agreement with Broth Microdilution D test Method
		Positive	Negative	
Staphylococcus aureus	9	3	6	100%
Coagulase Negative *Staphylococcus*	5	1	4	100%
Beta Haemolytic *Streptococcus* Group A and B	5	0	5	100%
Total	**19**	**4**	**15**	**100%**

CONCLUSION

The Sensititre 18-24 hour BMD D zone test, when compared to the CLSI M2 and M100 reference D zone agar disk approximation test demonstrated an equivalent level of performance when testing for inducible resistance to Clindamycin in macrolide resistant *Staphylococcus* spp. and *Streptococcus* spp.

- The Sensititre 18-24 hour dried susceptibility system had 100% agreement with reference methodology for all clinical isolates.
- The Sensititre 18-24 hour dried susceptibility system had 100% agreement with reference methodology for all challenge isolates.
- The Sensititre 18-24 hour dried susceptibility system had 100% agreement with reference methodology for all reproducibility isolates.
- The Sensititre 18-24 hour dried susceptibility system for BMD D zone testing would eliminate the need for additional off-line testing.
- The high level of agreement between the Sensititre 18-24 hour BMD D zone test and the CLSI reference method suggests the potential allure of this method for clinical laboratories.

REFERENCES

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