

# A Multi-Site Comparison Study of the Sensititre® Automated/Manual Susceptibility Plate to the NCCLS Microdilution Method for *Streptococcus pneumoniae* vs. Daptomycin

Authors: T. Kelley<sup>1\*</sup>, S. B. Killian<sup>1</sup>, C.C. Knapp<sup>1</sup>, N.Holliday<sup>1</sup>, M.R. Jacobs<sup>2</sup>, S.Bajaksouzian<sup>2</sup>, A.Windau<sup>2</sup>, J.A. Black<sup>3</sup>, E.S. Moland<sup>3</sup>  
 1. TREK Diagnostic Systems, Cleveland, OH 2. University Hospital, Cleveland, OH  
 3. Creighton University, Omaha, NE

## ABSTRACT

**Background:** A multi-site comparison was performed to evaluate the Sensititre susceptibility plate (TREK Diagnostic Systems, Cleveland, OH) for *Streptococcus pneumoniae* with Daptomycin (DAP) (Cubist Pharmaceuticals, Lexington, MA). Both automated and manual reading methodologies were tested against the frozen NCCLS reference broth microdilution method (BMD).  
**Methods:** Daptomycin (0.03-64µg/ml) was tested against 309 fresh clinical *Streptococcus pneumoniae*, 53 CDC challenge *Streptococcus pneumoniae*, and 25 reproducibility isolates. Sensititre plates were inoculated and read per the manufacture's instructions. BMD was performed in Muller-Hinton broth (supplemented with 2-5% lysed horse blood for pneumococci: NCCLS M7). The recommended NCCLS quality control organism *Streptococcus pneumoniae* ATCC 49619 was tested daily and performed within the specified range of 0.06-0.5µg/ml.  
**Results:** Comparisons of Daptomycin MIC results from the BMD method to both autoread and manually read Sensititre dried susceptibility plates were done. Percent essential agreement rates within +/- one log<sub>2</sub> dilution were determined. EA for the autoread method with the clinical isolates was 99.7% and the challenge isolates was 100%. EA for the manual read method with the clinical isolates was 99.7% and the challenge isolates was 100%. The 25 reproducibility isolates' EA for the automated and manual methods was 98.7% and 97.3%, respectively.  
**Conclusions:** This multi-site comparison of Daptomycin on the Sensititre susceptibility system for *Streptococcus pneumoniae*, gave reliable results using either the automated or manual read methodology compared to the reference BMD.

## PURPOSE OF THE STUDY

Daptomycin is a novel cyclic lipopeptide with cidal activity against the majority of gram positive isolates, including viridans group streptococci and *Streptococcus bovis*.<sup>3</sup> This study was to evaluate the performance of *Streptococcus pneumoniae* versus Daptomycin on the Sensititre® Dried susceptibility plate compared to the NCCLS microdilution reference method (M7 – A6).

## MATERIALS & METHODS

**Organisms:** The testing at 3 sites consisted of the following:  
 • 309 Clinical isolates  
 • 53 CDC Challenge isolates  
 • 25 Reproducibility isolates  
 • Quality Control strain, *Streptococcus pneumoniae* ATCC 49619

Antimicrobial		
Antimicrobial	Range Tested µg/ml	Supplied By
Daptomycin (DAP)	0.03-64µg/ml	Cubist Pharmaceuticals Lexington, MA

## SUSCEPTIBILITY TESTING METHODS

- Each *Streptococcus pneumoniae* was tested using a Sensititre dried susceptibility plate containing Daptomycin with a final calcium concentration of 50µg/ml. The plates were set-up and tested according to the manufacturer's instructions.
- The reference plates with Daptomycin, which requires 50µg/ml calcium were tested according to the microdilution methods published by the National Committee for Clinical Laboratory Standards (NCCLS, M7-A6).

## RESULTS

### Clinical Isolates vs. Daptomycin for *S. pneumoniae*

Read Method	Total Isolates Tested	Essential Agreement of Total	% Essential Agreement
Manual	309	308	99.7
Automated	309	308	99.7

### CDC Challenge Isolates vs. Daptomycin for *S. pneumoniae*

Read Method	Total Isolates Tested	Essential Agreement of Total	% Essential Agreement
Manual	53	53	100
Automated	53	53	100

### Combined Isolates vs. Daptomycin for *S. pneumoniae*

Read Method	Total Isolates Tested	Essential Agreement of Total	% Essential Agreement
Manual	362	361	99.7
Automated	362	361	99.7

### Reproducibility Isolates vs. Daptomycin for *S. pneumoniae* Essential Agreement Within +/- one log<sub>2</sub> Dilution of Reference MIC

Site	# Agreement Manual	# Agreement Autoread	Total Isolates Tested	Manual %	Autoread%
1	23	24	25	92	96
2	25	25	25	100	100
3	25	25	25	100	100
<b>Combined</b>	<b>73</b>	<b>74</b>	<b>75</b>	<b>97.3</b>	<b>98.7</b>

## RESULTS cont.

### *Streptococcus pneumoniae*, Manual Reading Method Distribution for Clinical Isolates vs. Daptomycin

		Reference Results												
		0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Test Results	0.03													
	0.06		2	1										
	0.12		1	98	35	1								
	0.25			49	138	8								
	0.5				19	4								
	1					1	5							
	2													
	4													
	8													
	16													
	32													
	64													
	>64													
TOTALS	0	3	148	192	14	5	0	0	0	0	0	0	0	

Essential Agreement of Total = 99.7% (361/362)

### *Streptococcus pneumoniae*, Automated Reading Method Distribution for Clinical Isolates vs. Daptomycin

		Reference Results												
		0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Test Results	0.03													
	0.06		2	4										
	0.12		1	97	39	1								
	0.25			47	135	8								
	0.5				18	4	1							
	1					1	3							
	2						1							
	4													
	8													
	16													
	32													
	64													
	>64													
TOTALS	0	3	148	192	14	5	0	0	0	0	0	0	0	

Essential Agreement of Total = 99.7% (361/362)

## CONCLUSION

This study compared the Sensititre® dried susceptibility plate with the NCCLS reference microdilution frozen plate (M7 – A6). The Sensititre dried susceptibility plate performed equivalent to the standard reference microdilution method.

**Clinical Isolates:**  
 The overall essential agreement for Daptomycin, within a +/- one log<sub>2</sub> dilution, was 99.7% for both the manual method and the autoread method.

**CDC Challenge Organisms:**  
 The overall essential agreement for Daptomycin, within a +/- one log<sub>2</sub> dilution, was 100% for both the manual method and the autoread method.

**Reproducibility:**  
 Interlaboratory reproducibility essential agreement was 97.3% for the manual read method and 98.7% for the autoread method.

## REFERENCES

- National Committee for Clinical Laboratory Standards. 2003. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-sixth edition*. Approved document M7-A6. Wayne, PA: NCCLS.
- National Committee for Clinical Laboratory Standards. 2005. *Performance standards for antimicrobial susceptibility testing, 14th informational supplement M100-S15*. Wayne, PA: NCCLS.
- Streit, JM, Steenbergen, JN, Thorne, GM, Alder, J, Jones, RN. Daptomycin tested against 915 bloodstream isolates of viridans group streptococci (eight species) and *Streptococcus bovis*. *Journal of Antimicrobial Chemotherapy* 2005.