Paper # **A-135**

An Evaluation of the Sensititre® Dried Susceptibility Plate Compared to the NCCLS (M7) Reference Broth Microdilution Method with the New Antimicrobial Agent Dalbavancin (Vicuron).

Authors: N. Holliday[1*], C.C. Knapp[1], S.B. Killian[1], T. Kelley[1] , and Beth P. Goldstein[2]

1. TREK Diagnostic Systems, Cleveland, OH
2. Vicuron Pharmaceuticals, King of Prussia, PA

TREK DIAGNOSTIC SYSTEMS

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

ABSTRACT

Background: Dalbavancin (Vicuron Pharmaceuticals King of Prussia, PA) is a novel glycopeptide antibiotic in late stage development for the treatment of gram-positive infections. An evaluation was undertaken to assess the performance of the Sensititre 18 - 24 hour dried susceptibility plate (manufactured by TREK Diagnostic Systems, Cleveland, OH) with dalbavancin. The Sensititre 18-24 hour dried susceptibility plate was tested along with the NCCLS (M7) reference broth microdilution method for comparison. The drug range tested for dalbavancin was (0.015 – 32 µg/ml). Linezolid (Pfizer New York, New York) was utilized as a comparator drug throughout performance testing.

Materials and Methods: The evaluation was conducted by Lab Services at TREK Diagnostic Systems with 442 isolates: 97 *Staphylococcus aureus*, 16 *Staphylococcus species*, 101 *Streptococcus pneumoniae*, 49 Beta Streps, 49 other *Streptococci species*, 61 *E. faecalis*, 34 *E. faecium*, 11 *Enterococcus species*, and 24 gram negative isolates. Ten reproducibility isolates were tested 3 times a day for 3 days. The recommended NCCLS and manufacturer's quality control organisms were tested daily, and were within the NCCLS expected quality control ranges for linezolid.

Results: Comparison of the Sensititre 18-24 hour dried susceptibility plate to the NCCLS (M7) reference broth microdilution method resulted (+/- one well) at 98% for dalbavancin and 100% for linezolid. Reproducibility was calculated as the percentage of results within (+/-) one dilution of the modal value. Overall agreement for the reproducibility of both dalbavancin and linezolid was 100%.

Conclusions: This evaluation indicates that the performance of the Sensititre 18-24 hour dried susceptibility plate with dalbavancin is equivalent to the NCCLS (M7) reference broth microdilution method.

PURPOSE OF THE STUDY

To evaluate the performance of Dalbavancin on the Sensititre 18 – 24 hour susceptibility plate compared to the NCCLS microdilution reference method (M7 – A6).

MATERIALS & METHODS

Organisms: The testing at TREK consisted of the following:

- 442 Total Clinical isolates
- 10 Reproducibility isolates
- 3 Quality Control strains

SUSCEPTIBILITY TESTING METHODS

- Each isolate was tested using a Sensititre 18 – 24 susceptibility plate. The plates were set-up and tested according to the manufacturers' instructions.
- The reference plate was tested according to the microdilution methods published by the National Committee for Clinical Laboratory Standards (NCCLS, M7-A6).

Antibiotics

Antimicrobial	Range Tested µg/ml	Supplied By
Dalbavancin (DAL)	0.015-32	Vicuron Pharmaceuticals
Linezolid (LZD)	0.015-32	Pfizer Pharmaceuticals

Recommended or Proposed QC Ranges for Dalbavancin/Linezolid

QC Organism	QC Range µg/ml (DAL)	QC Range µg/ml (LZD)	% Essential Agreement
E.faecalis 29212	0.03-0.12	1-4	100
S.aureus 29213	0.03-0.12	1-4	100
S.pneumoniae 49619	0.008-0.03	0.5-2	100

Organism Species	Number of Isolates Tested	% Essential Agreement Dalbavancin	% Essential Agreement Linezolid
Staphylococcus aureus	97	98%	100%
Staphylococcus species	16	100%	100%
Streptococcus pneumoniae	101	99.5%	100%
Beta Streps	49	94%	100%
Other *Streptococci species*	49	100%	100%
Enterococcus faecalis	61	94%	100%
Enterococcus faecium	34	100%	100%
Enterococcus species	11	100%	100%
Gram Negative isolates	24	100%	100%
Total	**442**	**98%**	**100%**

RESULTS

Staphylococcus aureus* and *Staphylococcus species
MIC Distribution for Clinical Isolates vs. Dalbavancin

Test Results (Dried) \ NCCLS Reference Results (Frozen)	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
≤0.015	4												
0.03	3	15	2										
0.06		14	51	1									
0.12		2	12	3									
0.25				2	2								
0.5						1							
1							1						
2													
4													
8													
16													
32													
>32													
TOTALS	**7**	**31**	**65**	**6**	**2**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**

% Essential Agreement of Total= 99%

Streptococcus pneumoniae*, Beta Streps and other *Streptococcus species
MIC Distribution for Clinical Isolates vs. Dalbavancin

Test Results (Dried) \ NCCLS Reference Results (Frozen)	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
≤0.015	73	7											
0.03	50	40	1										
0.06	4	14	9	1									
0.12													
0.25													
0.5													
1													
2													
4													
8													
16													
32													
>32													
TOTALS	**127**	**61**	**10**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

% Essential Agreement of Total= 98%

Reproducibility Results for Dalbavancin

Organisms	Plate	Day 1 Replicates			Day 2 Replicates			Day 3 Replicates			Mode
		1	2	3	1	2	3	1	2	3	
E.faecalis 19-6848A	Test	0.06	0.06	0.06	0.03	0.06	0.03	0.03	0.03	0.06	0.06
	Reference	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
E.faecalis 29212	Test	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
	Reference	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
S.aureus 43-507D	Test	0.12	0.12	0.12	0.06	0.06	0.06	0.06	0.06	0.06	0.06
	Reference	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
*CNS 1-6777A	Test	0.03	0.06	0.06	0.06	0.06	0.03	0.03	0.03	0.03	0.03
	Reference	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
S.aureus 25923	Test	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
	Reference	0.06	0.06	0.06	0.06	0.06	0.12	0.06	0.06	0.06	0.06
S.aureus 29213	Test	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
	Reference	0.06	0.06	0.06	0.06	0.06	0.06	0.03	0.03	0.03	0.06
*SPN 49619	Test	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015
	Reference	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015
*SPN 13-3602	Test	0.03	0.03	0.03	<=0.015	0.03	0.03	<=0.015	<=0.015	<=0.015	0.03
	Reference	0.03	0.03	0.03	<=0.015	0.03	0.03	<=0.015	<=0.015	<=0.015	0.03
*VgS 15-6797	Test	0.03	0.03	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015
	Reference	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015
*BSA 7-6560A	Test	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015
	Reference	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015	<=0.015

% Agreement for Total =100% (+/- one well of Modal Value)

*SPN-*Streptococcus pneumoniae*
*CNS-Coagulase Negative *Staphylococcus*
*BSA-Beta Strep
*Vgs- Strep Viridans

Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus species
MIC Distribution for Clinical Isolates vs. Dalbavancin

Test Results (Dried) \ NCCLS Reference Results (Frozen)	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
≤0.015													
0.03		4	8										
0.06		5	41	17	1								
0.12			4	8	2								
0.25													
0.5													
1							1	1					
2													
4													1
8										1			
16											3	3	
32											1	3	
>32													2
TOTALS	**0**	**9**	**53**	**25**	**3**	**1**	**1**	**0**	**0**	**1**	**4**	**6**	**3**

% Essential Agreement of Total= 98%

CONCLUSION

This investigation compared the 18 – 24 hour Sensititre susceptibility plate with the NCCLS reference microdilution frozen plate (M7 – A6). The Sensititre plate is equivalent to the NCCLS reference method.

The overall essential agreement for Dalbavancin, within a +/- one-well dilution range, was 98%.

The overall essential agreement for Linezolid, within a +/- one-well dilution range, was 100%.

Overall agreement for the reproducibility (+/- one well of the modal value) of both Dalbavancin and the comparator Linezolid was 100%.

Overall essential agreement for the QC organisms was 100%. All QC organisms were within recommended NCCLS ranges for both Dalbavancin and Linezolid.

Since there were no interpretive criteria for Dalbavancin, gram negative isolates were used to test the performance of the complete dilution range on the dried and frozen plates.