Paper # **C-125**

An Evaluation of the Sensititre® Dried Susceptibility Plate compared with CLSI (M7) Reference Broth Microdilution Method with the New Antimicrobial Agent, Retapamulin

N.HOLLIDAY,[1] C.C. KNAPP,[1] S.B. KILLIAN,[1] and N.E. SCANGARELLA[2]
1.TREK Diagnostic Systems, Cleveland, OH, USA; 2.GlaxoSmithKline, Collegeville, PA, USA

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

ABSTRACT

Background: Retapamulin (RE, SB-275833), a novel pleuromutilin with a unique mode of action and no target specific cross-resistance to other antibacterials, is being developed for the topical treatment of uncomplicated skin infections. As with any new agent it is critical to establish performance of the commonly used, dried susceptibility panel to determine its accuracy and reproducibility compared with reference methods. An evaluation was undertaken to assess performance of the Sensititre 18–24 h dried susceptibility plate (TREK Diagnostic Systems, Cleveland, OH) for RE.
Methods: Two hundred isolates (20 Coagulase Negative *Staphylococcus* spp., 60 *S. aureus*, 60 Beta *Streptococcus* spp., 20 *Enterococcus* spp., 20 Enterobacteriaceae, and 20 Non-Enterobacteriaceae) were tested with RE (0.002–256 g/ml) and 6 comparators using Sensititre 18–24 h dried susceptibility plate and by CLSI (NCCLS; M7) reference broth microdilution method for comparison. For reproducibility testing, 10 isolates were tested 3 times daily 3 days. Recommended CLSI quality control (QC) organisms were tested daily and were within the CLSI expected QC ranges for all comparators. RE results for the standard QC organisms were within the ranges established by the manufacturer.
Results: Comparison of the Sensititre plate to the CLSI (M7) reference broth microdilution method resulted in 100% essential agreement (+/- one log2 dilution) for RE and all comparator drugs. Reproducibility was calculated as the percentage of results within (+/-) one log2 dilution of the modal value. Overall agreement for the reproducibility of RE and all comparator drugs was 100%.
Conclusions: This evaluation indicates that the performance of the Sensititre 18–24 hour dried susceptibility plate with RE is equivalent to the CLSI (M7) reference broth microdilution method and is an acceptable method for susceptibility testing of RE.*

*For Research Use Only. Not for Use in Diagnostic Procedures.

PURPOSE OF THE STUDY

To evaluate the performance of **Retapamulin**, (SB-275833) a novel pleuromutilin, on the dried Sensititre 18 – 24 hour susceptibility plate compared to the CLSI reference broth microdilution method (M7 – A6).

MATERIALS & METHODS

Organisms Tested

• **200** Clinical and Challenge isolates consisting of :

Organism	Number of Isolates Tested
Coagulase-negative staphylococci	20
Staphylococcus aureus	60
Beta-hemolytic streptococci	60
Enterococcus spp.	20
Enterobacteriaceae	20
Non-Enterobacteriaceae	20

• **10** Reproducibility isolates with on-scale endpoints

Organisms Tested
Methicillin Resistant *Staphylococcus aureus*
Methicillin Susceptible *Staphylococcus aureus*
Methicillin Susceptible Coagulase Negative *Staphylococcus* spp.
Methicillin Susceptible Coagulase Negative *Staphylococcus* spp.
Beta-hemolytic *Streptococcus* group A
Beta-hemolytic *Streptococcus* group B
Enterococcus. faecalis
Streptococcus. pneumoniae ATCC 49619
Staphylococcus aureus ATCC 29213
Enterococcus faecalis ATCC 29212

• CLSI quality control strains (20 replicates of each tested)

Organism	ATCC Number
Staphylococcus aureus	ATCC 29213
Enterococcus faecalis	ATCC 29212
Streptococcus pneumoniae	ATCC 49619
Escherichia coli	ATCC 25922
Escherichia coli	ATCC 35218
Pseudomonas aeruginosa	ATCC 27853

MATERIALS & METHODS cont.

Antimicrobials Tested

Antimicrobials Tested	Range Tested ug/ml	Supplied By
Retapamulin **(SB-275833)**	0.002-256	GlaxoSmithKline(GSK) Collegeville, PA
Gentamicin	0.03-64	Sigma-Aldrich St. Louis, Missouri
Erythromycin	0.015-32	Sigma-Aldrich St. Louis, Missouri
Tetracycline	0.06-32	Sigma-Aldrich St. Louis, Missouri
Linezolid	0.015-16	Pfizer Pharmaceuticals New York, New York
Amoxicillin/Clavulanic acid	0.015/0.008-32/16	Sigma-Aldrich St. Louis, Missouri / GSK Collegeville, PA
Cephalothin	0.03-256	Sigma-Aldrich St. Louis, Missouri

SUSCEPTIBILITY TESTING METHODS

- Each isolate was tested using a Sensititre 18 – 24 h susceptibility plate containing **Retapamulin** and comparator antimicrobials. The plates were set-up and tested according to the manufacturers' instructions.
- The reference broth microdilution plates were prepared and tested according to the Clinical Laboratory Standards Institute (CLSI, M7-A6).
- Repeat testing was performed for any organism/antimicrobial combination that resulted in a greater than +/- one $\log_2$ dilution error. These were repeated in triplicate on both the dried test plate and reference plate.
- Reproducibility testing: 10 Clinical/Challenge strains that had on-scale endpoints, (chosen from the 200 isolates) including the CLSI ATCC QC isolates, were tested in triplicate on 3 separate days. Any results on reproducibility testing with a greater than +/- one $\log_2$ dilution difference were retested as per the criteria for repeat testing.

RESULTS

Essential agreement for the 200 isolates were calculated using the +/- one $\log_2$ dilution standard for comparison studies. The agreement rates for the 200 isolates were as follows:

Antimicrobials	# Isolates TESTED	% Essential Agreement (B/A)*
Retapamulin	200	100
Gentamicin	200	100
Erythromycin	200	99/100
Tetracycline	200	100
Linezolid	200	100
Cephalothin	200	100
Amox/Clav	200	100

*(B/A) indicates before/after retesting

Reproducibility results for **Retapamulin** and the comparator antimicrobials within +/- one $\log_2$ dilution of the CLSI reference broth microdilution plate.

Antimicrobials	# Isolates TESTED	% Essential Agreement (B/A)*
Retapamulin	10	100
Gentamicin	10	100
Erythromycin	10	100
Tetracycline	10	100
Linezolid	10	100
Cephalothin	10	100
Amox/Clav	10	100

*(B/A) indicates before/after retesting

RESULTS cont.

***Staphylococcus* spp.**
MIC Distribution for Clinical Isolates vs. Retapamulin

Test Results (Dried) \ CLSI Reference Results (Frozen)	≤0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	>4
≤0.002													
0.004													
0.008													
0.015													
0.03					3	2							
0.06						35	1						
0.12						5	30						
0.25							1						
0.5								1					
1									1				
2												1	
4													
>4													
TOTALS	0	0	0	0	3	42	32	0	1	1	0	1	0

% Essential Agreement of Total =100%

Enterobacteriaceae/Non-Enterobacteriaceae
MIC Distribution for Clinical Isolates vs. Retapamulin

Test Results (Dried) \ CLSI Reference Results (Frozen)	≤0.25	0.25	0.05	1	2	4	8	16	32	64	128	256	>256
≤0.25													
0.25													
0.05													
1													
2													
4													
8								1					
16								9					
32										1			
64										14	2		
128											3	1	
256												2	
>256													7
TOTALS	0	0	0	0	0	0	0	10	0	15	5	3	7

% Essential Agreement of Total =100%

Reproducibility Results for Retapamulin

Organisms	Plate	Day 1 Replicates			Day 2 Replicates			Day 3 Replicates			Mode
		1	2	3	1	2	3	1	2	3	
MRSA	Test	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
	Reference	0.12	0.06	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
MSSA	Test	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
	Reference	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
MSCNS	Test	0.03	0.03	0.03	0.03	0.03	0.03	0.06	0.03	0.06	0.03
	Reference	0.03	0.03	0.03	0.03	0.03	0.03	0.06	0.06	0.06	0.03
MSCNS	Test	0.12	0.12	0.12	0.06	0.12	0.12	0.12	0.12	0.12	0.12
	Reference	0.06	0.06	0.06	0.06	0.06	0.06	0.12	0.12	0.12	0.06
BSA	Test	0.03	0.03	0.03	0.03	0.03	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
BSB	Test	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
	Reference	0.03	0.03	0.03	0.03	0.03	0.03	0.06	0.06	0.06	0.03
EF	Test	128	128	128	128	128	128	128	128	128	128
	Reference	128	128	128	128	128	128	128	128	128	128
SPN	Test	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
49619	Reference	0.25	0.25	0.25	0.25	0.25	0.25	0.12	0.12	0.12	0.25
SA	Test	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
29213	Reference	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12

RESULTS cont.

***Enterococcus* spp.**
MIC Distribution for Clinical Isolates vs. Retapamulin

Test Results (Dried) \ CLSI Reference Results (Frozen)	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
≤0.03	1												
0.06	1	6											
0.12			3										
0.25													
0.5													
1							1						
2								1					
4													
8													
16													
32													
64												1	
>64													6
TOTALS	2	6	3	0	0	0	1	1	0	0	0	1	6

% Essential Agreement of Total =100%

Beta-hemolytic streptococci
MIC Distribution for Clinical Isolates vs. Retapamulin

Test Results (Dried) \ CLSI Reference Results (Frozen)	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
≤0.03	18												
0.06	2	38											
0.12													
0.25				1									
0.5													
1						1							
2													
4													
8													
16													
32													
64													
>64													
TOTALS	20	38	0	1	0	1	0	0	0	0	0	0	0

% Essential Agreement of Total =100%

CONCLUSION

The Sensititre 18-24 hour dried susceptibility plate demonstrated an equivalent level of performance to the CLSI, M7 reference broth microdilution plate for susceptibility testing of Retapamulin.

- The overall essential agreement for **Retapamulin,** within +/- one log2 dilution range, was 100% using the manual method.
- The overall essential agreement for all comparators, within +/- one log2 dilution range, was 100% using the manual method.
- Interlaboratory reproducibility was 100% for **Retapamulin** and all comparator drugs.

REFERENCES

1. Clinical and Laboratory Standards Institute. 2006. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-sixth edition.* Approved document M7-A7. Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute. 2006. *Performance standards for antimicrobial susceptibility testing, 16th informational supplement* M100-S16. Wayne, PA: CLSI.