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An Evaluation of the Sensititre® MIC Susceptibility System Compared to the CLSI (M31) Reference Broth Microdilution Method with the New Antimicrobial Agent, Cefovecin

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ABSTRACT

Background: Cefovecin (FOV) (Pfizer Animal Health, Kalamazoo, MI), is a new semi-synthetic extended-spectrum cephalosporin antibiotic. It is indicated for treatment of bacterial infections in small animals. An evaluation was undertaken to determine the accuracy and reproducibility of the Sensititre 18 to 24-hour dried susceptibility system (manufactured by TREK Diagnostic Systems, Cleveland, OH) with Cefovecin, compared to the CLSI (M31) reference broth microdilution method (BMD). The range tested for Cefovecin was 0.0005 – 32 µg/ml on both the Sensititre and BMD plates.
Materials and Methods: Two hundred isolates (20 *Staphylococcus intermedius,* 20 *Staphylococcus aureus*, 20 *Streptococcus canis (group G),* 20 *Escherichia coli,* 20 *Proteus mirabilis,* 20 *Pasteurella multocida,* 20 *Histophilus somnus,* 20 *Actinobacillus pleuropneumoniae,* 20 *Bacteroides fragilis* and 20 *Prevotella* species*)* were tested with FOV and 3 comparators, Cefpodoxime, Ceftiofur, and Cefoxitin, using the Sensititre 18-24 h dried susceptibility plate and by CLSI (M31) BMD for comparison. For reproducibility testing, 10 isolates were tested 3 times daily over a period of three days. Recommended CLSI quality control (QC) organisms were tested daily and were within the CLSI expected quality control ranges for FOV and all comparators.
Results: Comparison of the Sensititre plate to the CLSI (M31) BMD resulted in 100% essential agreement (+/- one $\log_2$ dilution) for FOV and 100% for comparators. Reproducibility was calculated as the percentage of results within +/- one $\log_2$ dilution of the modal value. Overall agreement for the reproducibility of FOV and for comparators was 100%.
Conclusions: This evaluation indicates that the Sensititre 18 to 24-hour dried susceptibility system with FOV is equivalent to the CLSI (M31) BMD and is an acceptable method for susceptibility testing of FOV.

PURPOSE OF THE STUDY

To evaluate the performance of Cefovecin, an extended-spectrum cephalosporin antibiotic, on the Sensititre 18 – 24 hour susceptibility system compared to the CLSI reference broth microdilution method (M31).

MATERIALS & METHODS

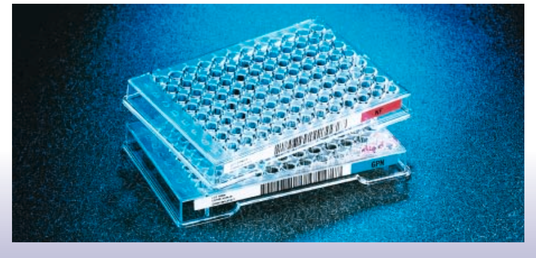
Organisms Tested

• 200 Clinical and Challenge isolates consisting of:

Organism	Number of Isolates Tested
Staphylococcus intermedius	20
Streptococcus canis (grp G)	20
Escherichia coli	20
Proteus mirabilis	20
Pasteurella multocida	20
Histophilus somnus	20
Actinobacillus pleuropneumoniae	20
Bacteriodes fragilis (group)	20
Prevotella species	20

• 10 Reproducibility isolates with on-scale endpoints

Organisms Tested
Actinobacillus pleuropneumoniae
Actinobacillus pleuropneumoniae ATCC 27090
Streptococcus canis
Streptococcus pneumoniae ATCC 49619
Streptococcus intermedius
Bacteriodes fragilis ATCC 25285
Prevotella species
Staphylococcus aureus ATCC 29213
Escherichia coli
Escherichia coli ATCC 25922



MATERIALS & METHODS cont.

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

Organism	ATCC Number
Staphylococcus aureus	ATCC 29213
Histophilus somnus	ATCC 700025
Actinobacillus pleuropneumoniae	ATCC 27090
Streptococcus pneumoniae	ATCC 49619
Escherichia coli	ATCC 25922
Eubacterium lentum	ATCC 43055
Bacteriodes fragilis	ATCC 25285
Bacteriodes thetaiotaomicron	ATCC 29741

*Daily Quality Control isolates were run on both the reference and dried plates each test day.

Antimicrobials Tested

Antimicrobials Tested	Range Tested µg/ml	Supplied By
Cefovecin	0.0005-32	Pfizer Animal Health Kalamazoo, MI
Cefpodoxime	0.015-32	Sankyo, Japan
Ceftiofur	0.00025-0.25	Pfizer Groton, CT
Cefoxitin	0.25-256	Sigma-Aldrich Saint Louis, MO

SUSCEPTIBILITY TESTING METHODS

- Each isolate was tested using a custom dried Sensititre 18 – 24h susceptibility plate containing Cefovecin and comparator drugs. The plates were set up according to manufacturers' instructions.
- The CLSI reference broth microdilution plates were prepared and tested according to the Clinical Laboratory Standards Institute (CLSI- M31).
- Repeat testing was performed if any organism/antimicrobial combination resulted in a greater then +/- one $\log_2$ dilution error. These would be repeated in triplicate on both the dried test plate and the reference plate.
- Reproducibility: 10 Clinical/ challenge strains that had on-scale endpoints, (chosen from the 200 previously chosen isolates) including the CLSI ATCC QC isolates, were tested in triplicate on 3 separate days. Any results on reproducibility with a greater then +/- one $\log_2$ dilution difference would also be re-tested as per the criteria for repeat testing.

RESULTS

Staphylococcus aureus, Staphylococcus intermedius

CLSI Reference Results (Frozen) / Test Results (Dried)

Test Results (Dried) \ CLSI Reference Results (Frozen)	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
≤0.03												
0.06												
0.12		5	3									
0.25			1									
0.5				4	1							
1					5	6						
2						1	1					
4							3					
8								1				
16									1			
32												
>32												8
TOTALS	0	5	4	4	6	7	4	0	1	1	0	8

Total Isolates 40 — Essential Agreement of Evaluable 32
Total Evaluable Isolates 32 — % Essential Agreement of Total 100%
Essential Agreement of Total 40 — % Essential Agreement of Evaluable 100%

RESULTS cont.

Histophilus somnus, Actinobacillus pleuropneumoniae

CLSI Reference Results (Frozen) / Test Results (Dried)

Test Results (Dried) \ CLSI Reference Results (Frozen)	≤0.0005	0.001	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	>0.5
≤0.0005												
0.001												
0.002			5									
0.004			12	1								
0.008				1								
0.015					4	6						
0.03						7	2					
0.06							1	1				
0.12												
0.25												
0.5												
>0.5												
TOTALS	0	0	17	2	4	13	3	1	0	0	0	0

Total Isolates 40 — Essential Agreement of Evaluable 40
Total Evaluable Isolates 40 — % Essential Agreement of Total 100%
Essential Agreement of Total 40 — % Essential Agreement of Evaluable 100%

***Bacteriodes fragilis, Prevotella* species**

CLSI Reference Results (Frozen) / Test Results (Dried)

Test Results (Dried) \ CLSI Reference Results (Frozen)	≤0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
≤0.12	1											
0.25	6	7										
0.5		7										
1			2									
2												
4						4	1					
8							8	1				
16												
32												
64												
128												
>128											3	
TOTALS	7	14	2	0	0	4	9	1	0	0	3	0

Total Isolates 40 — Essential Agreement of Evaluable 36
Total Evaluable Isolates 36 — % Essential Agreement of Total 100%
Essential Agreement of Total 40 — % Essential Agreement of Evaluable 100%

Escherichia coli, Proteus mirabilis

CLSI Reference Results (Frozen) / Test Results (Dried)

Test Results (Dried) \ CLSI Reference Results (Frozen)	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
≤0.03												
0.06	2	1										
0.12		9	1									
0.25			6	2								
0.5				3	2							
1					9	3						
2						1						
4							1					
8												
16												
32												
>32												
TOTALS	2	10	7	5	11	4	1	0	0	0	0	0

Total Isolates 40 — Essential Agreement of Evaluable 40
Total Evaluable Isolates 40 — % Essential Agreement of Total 100%
Essential Agreement of Total 40 — % Essential Agreement of Evaluable 100%

Streptococcus canis, Pasteurella multocida

CLSI Reference Results (Frozen) / Test Results (Dried)

Test Results (Dried) \ CLSI Reference Results (Frozen)	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
≤0.03	25											
0.06	12											
0.12		2	1									
0.25												
0.5												
1												
2												
4												
8												
16												
32												
>32												
TOTALS	37	2	1	0	0	0	0	0	0	0	0	0

Total Isolates 40 — Essential Agreement of Evaluable 15
Total Evaluable Isolates 15 — % Essential Agreement of Total 100%
Essential Agreement of Total 40 — % Essential Agreement of Evaluable 100%

RESULTS cont.

Reproducibility

Isolate	Day 1 TEST FOV			Day 2 TEST FOV			Day 3 TEST FOV			MODE
	A	B	C	A	B	C	A	B	C	
A. pleuropneumoniae	0.015	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
A. pleuropneumoniae 27090	0.015	0.015	0.015	0.015	0.03	0.03	0.015	0.03	0.015	0.015
S. canis	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
S. pneumoniae 49619	0.25	0.25	0.25	0.25	0.25	0.25	0.12	0.12	0.12	0.25
S. intermedius	1	1	1	1	1	1	1	1	1	1
S. aureus 29213	1	1	1	1	1	1	1	1	1	1
E. coli	1	1	1	1	1	1	1	1	2	1
E. coli 25922	1	1	1	1	1	1	1	1	1	1
Prevotella spp.	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
B. fragilis 25285	8	16	8	8	8	8	16	8	8	8

Isolate	Day 1 REFERENCE FOV			Day 2 REFERENCE FOV			Day 3 REFERENCE FOV			MODE
	A	B	C	A	B	C	A	B	C	
A. pleuropneumoniae	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
A. pleuropneumoniae 27090	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
S. canis	<=0.03	<=0.03	<=0.03	0.06	0.06	0.06	0.06	0.06	0.06	0.06
S. pneumoniae 49619	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
S. intermedius	1	1	1	1	1	1	0.5	1	1	1
S. aureus 29213	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
E. coli	1	1	1	1	1	1	1	1	1	1
E. coli 25922	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Prevotella spp.	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	<=0.12	0.25
B. fragilis 25285	8	8	8	8	8	8	8	8	8	8

CONCLUSION

The Sensititre 18-24 hour dried susceptibility system demonstrated an equivalent level of performance to the CLSI, M31 reference broth microdilution plate for susceptibility testing of **Cefovecin**.

Isolates Tested:
- The overall essential agreement for **Cefovecin**, within a +/- one $\log_2$ dilution range, was 100% using the manual method.
- The overall essential agreement for comparators, within a +/- one $\log_2$ dilution range, was 100% using the manual method.

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	No. Isolates Tested	% Essential Agreement
Cefovecin	200	100%
Cefpodoxime	200	100%
Ceftiofur	200	100%
Cefoxitin	200	100%

Reproducibility:
- Interlaboratory reproducibility was 100% for **Cefovecin** and all comparator drugs.

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

Antimicrobials	No. Isolates Tested	% Essential Agreement
Cefovecin	10	100%
Cefpodoxime	10	100%
Ceftiofur	10	100%
Cefoxitin	10	100%

This study validates that the Sensititre Dried Susceptibility system's performance is equivalent to the CLSI M31 reference microdilution method

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