Development of Tebipenem MIC Antimicrobial Susceptibility Test for Gram-negative Bacteria on MicroScan Dried Gram-negative MIC Panels

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ABSTRACT

Background: Development of a tebipenem antimicrobial susceptibility test was completed for the MicroScan Dried Gram-negative MIC (MSDGN) Panel when compared to CLSI broth microdilution reference panels.
Materials/Methods: Development was conducted by comparing MICs obtained using the MSDGN panel to MICs using a CLSI broth microdilution reference panel. A total of 223 Enterobacterales isolates were tested in triplicate for a total of 669 results with turbidity and Prompt®* methods of inoculation. MSDGN panels were incubated at 35±2ºC and read on the WalkAway System, the autoSCAN-4 instrument, and read visually. Frozen reference panels, prepared according to CLSI methodology, were inoculated using the turbidity inoculation method. All frozen reference panels were incubated at 35±2ºC and read visually. Dilution sequence evaluated is 0.03-32 µg/mL.
Results: When compared to frozen reference panel results, essential agreement for all isolates tested during development are as follows:

Read Method	Essential Agreement %	
	Turbidity	Prompt
Visual	97.6 (653/669)	94.2 (630/669)
WalkAway	97.2 (650/669)	94.3 (631/669)
autoSCAN-4	94.0 (629/669)	93.7 (627/669)

Conclusion: The development data showed that tebipenem MIC results for Enterobacterales obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels. Essential agreement is >90% for all inoculation and read methods.

For investigational Use Only. The performance characteristics of this product have not been established.

INTRODUCTION

MicroScan Dried Gram Negative MIC panels were developed for testing of Gram Negative bacteria with tebipenem (TBP). Tebipenem is a carbapenem targeting complicated urinary tract infections against clinically important *Enterobacterales* spp. including ESBL and AmpC-β-lactamase-producing organisms. Data from a development study at Beckman Coulter evaluated the performance of a MicroScan Dried Gram Negative MIC panel with tebipenem using *Enterobacterales* isolates.

METHODS

Development Study Design:
MicroScan Dried Gram Negative MIC panels were tested concurrently with a CLSI frozen broth microdilution reference panel using both the turbidity and Prompt Inoculation methods.
A total of 223 Enterobacterales spp. isolates were set up in triplicate for a total of 669 results.

METHODS (Continued)

Quality Control Expected Results, CLSI M100-ED31
Escherichia coli ATCC 25922: 0.008 – 0.03 µg/ml*
Pseudomonas aeruginosa ATCC 27853: 1 - 8 µg/ml
*extrapolated to validation panel dilutions

Panels
Frozen reference and MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of tebipenem 0.03 - 32 µg/ml in cation-adjusted Mueller-Hinton broth. Reference panels were prepared and frozen following CLSI recommendations.

Quality Control
Quality control (QC) testing was performed daily using ATCC 25922 *E. coli* and ATCC 27853 *P. aeruginosa* (CLSI M100-ED31).

Panel Inoculation, Incubation, and Reading
All isolates were subcultured onto trypticase soy agar (TSA) with 5% sheep blood and incubated for 18-24 hours at 35±2ºC ºC prior to testing. Isolates from frozen stocks were subcultured twice before testing.
Inoculum suspensions for each strain were prepared with the direct standardization (turbidity standard) method for MSDGN MIC and frozen reference panels. MSDGN MIC panels were also inoculated using the Prompt Inoculation method.
Following inoculation, MSDGN MIC panels were incubated at 35± 2ºC in the WalkAway system for 16-20 hours. Frozen reference panels were incubated in an off-line incubator. All dried panels were read by the WalkAway, autoSCAN-4, and manually.

Data Analysis
Essential Agreement (EA) = MSDGN panel MIC within +/- 1 dilution of the frozen reference result MIC.
Categorical Agreement (CA) was not calculated because no breakpoints yet.
Bias = For all testing, a bias ≤30% is considered indicative of random variation. The following calculation is applied to the overall data: |(% test results above reference)–(% test results below references)|
MIC distribution of the reference results will be presented.

RESULTS

Efficacy (Table 1)
A total of 669 *Enterobacterales* spp., isolates were tested at 16, 18, 20hrs.

Table 1. Efficacy - *Enterobacterales*
Performance for *Enterobacterales* in the combined phases of efficacy and challenge are as follows with the Prompt inoculation (P) or turbidity inoculation (T):

Read Method	Inoc Method	Essential Agreement		Bias	
		No.	%	%	Trend
WalkAway	P	631/669	94.3	2.1	No Bias
autoSCAN-4		627/669	93.7	6.1	No Bias
Visual		630/669	94.2	14.3	No Bias
WalkAway	T	650/669	97.2	5.4	No Bias
autoSCAN-4		629/669	94.0	16.4	No Bias
Visual		653/669	97.6	4.2	No Bias

MIC distribution (table2), 669 frozen reference MIC results collected at 16, 18, and 20hrs.

Table 2. Frozen Reference MIC distribution

By Species	<=0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32	Total
Citrobacter freundii complex	16	2		3			2	6	4	3			36
Enterobacter aerogenes	18	12	2	1									33
Enterobacter cloacae	45	25	21	6	5			3			3		108
Escherichia coli	90	10	5	3									108
Klebsiella oxytoca	28	2	3										33
Klebsiella pneumoniae	52	17	3				2	6	1		6	21	108
Morganella morganii			2	26	20								48
Proteus mirabilis	2	9	45	43	8	1							108
Providencia rettgeri			10	2									12
Providencia stuartii	2	4		4	2								12
Salmonella species	21												21
Serratia marcescens	2	6	8	5									21
Shigella species	20		1										21
Total	296	87	100	93	35	1	4	15	5	3	9	21	669

Quality Control (Table 6)
QC results for the frozen reference panel were 100% in range for ATCC 25922 *E. coli* and 100% in range for ATCC 27853 *P. aeruginosa*.

Table 6. Quality Control

Organism	QC Range (mg/L)	WalkAway		autoSCAN-4		Manual	
		Prompt	Turbidity	Prompt	Turbidity	Prompt	Turbidity
E. coli ATCC 25922	0.008-0.03	100%	100%	97.7%	100%	97.7%	100%
P. aeruginosa ATCC 27853	1-8	100%	100%	100%	100%	100%	100%

CONCLUSION

The development data showed that tebipenem MIC results for Enterobacterales obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels. Essential agreement is >90% for all inoculation and read methods.

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