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

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ABSTRACT[1]

Background: Development of a cefepime-taniborbactam (FTB) antimicrobial susceptibility test was completed for the MicroScan Dried Gram-negative MIC (MSDGN) Panel 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 1376 isolates (1256 Enterobacterales isolates and 120 *Pseudomonas aeruginosa* isolates) were tested at 16-, 18-, and 20-hour incubation times using the turbidity and Prompt®* methods of inoculation. MSDGN panels were incubated at 35 ± 1ºC and read on the WalkAway System, the autoSCAN-4 instrument, and read visually. Frozen reference panels, prepared according to ISO/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 0.12/4-64/4 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	98.8 (1359/1376)	98.0 (1349/1376)
WalkAway	98.2 (900/917)	93.0 (853/917)
autoSCAN-4	98.2 (1351/1376)	95.6 (1316/1376)

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

[1]Pending submission and clearance by the United States Food and Drug Administration; not yet available for in vitro diagnostic use in the US. 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 cefepime-taniborbactam. Cefepime-taniborbactam is a novel investigational antibacterial agent for the treatment of multidrug-resistant Enterobacterales and *P. aeruginosa* including strains producing Ambler class A, B, C, and D β-lactamases[1,2]. Cefepime-taniborbactam was statistically superior to meropenem in a Phase 3 study of adults with complicated urinary tract infections[3]. Data from a development study at Beckman Coulter evaluated the performance of a MicroScan Dried Gram-Negative MIC panels with cefepime-taniborbactam using Enterobacterales and *P. aeruginosa* isolates.

METHODS

Development Study Design:
MicroScan Dried Gram-Negative MIC panels were tested concurrently with a CLSI frozen broth microdilution reference[4] panel using both the turbidity and Prompt Inoculation methods.
Isolates were set up in triplicate and incubated at 16-, 18-, and 20-hours. WalkAway results do not include 20-hour incubation.

Panel builds
Frozen reference and MicroScan Dried Gram-Negative MIC panels contained two-fold doubling dilutions of cefepime-taniborbactam 0.12/4 – 64/4 µg/ml in cation-adjusted Mueller-Hinton broth. Reference panels were prepared and frozen following CLSI M07 recommendations.

Quality Control and Ranges (µg/mL) per CLSI M100-ED32[5]
Quality control (QC) testing was performed daily using
ATCC 25922 *E. coli* ATCC 35218 E. coli 0.016/4 – 0.06/4
NCTC 13353 *E. coli* 0.12/4 – 1/4
ATCC 700603 *K. pneumoniae* 0.12/4 – 0.5/4
ATCC BAA-1705 *K. pneumoniae* 0.12/4 – 0.5/4
ATCC 27853 *P. aeruginosa* 0.5/4 – 4/4

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 34-37ºC 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±1ºC 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 evaluated because there are no CLSI, FDA or EUCAST breakpoints.
Bias = For all isolates 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), results for Enterobacterales and *P. aeruginosa* met acceptance criteria with Prompt and turbidity.

Table 1. Efficacy
Development performance with the Prompt inoculation (P) and turbidity inoculation (T):

Read Method	Inoculation Method	Essential Agreement		Bias	
		No.	%	%	Trend
WalkAway	P	953/917	93.0	1.7	No bias
autoSCAN-4	P	1316/1376	95.6	8.1	No bias
Visual	P	1349/1376	98.0	10.6	No bias
WalkAway	T	900/917	98.2	7.5	No bias
autoSCAN-4	T	1351/1376	98.2	11.2	No bias
Visual	T	1359/1376	98.8	9.4	No bias

Quality Control (Table 3), QC results for the test dried panel met acceptance criteria.

Table 3. Quality Control
Cefepime-taniborbactam Dried test panel results with Prompt and turbidity inoculation methods,

Organism	WalkAway		autoSCAN-4		Manual	
	Prompt (%)	Turbidity (%)	Prompt (%)	Turbidity (%)	Prompt (%)	Turbidity (%)
ATCC 25922	100	100	100	100	100	100
ATCC 35218	100	95.2	100	100	100	100
NCTC 13353	95.5	100	96.9	100	96.9	96.9
ATCC 700603	100	100	100	100	100	100
ATCC BAA-1705	100	95.5	100	100	100	100
ATCC 27853	100	100	100	100	100	100

Frozen Reference MIC distribution (Table 2), cefepime-taniborbactam frozen reference MIC results collected from 16-, 18-, and 20-hours incubation.
Table 2. Frozen Reference MIC distribution

Dilutions (µg/mL) / By Species	<=0.12	0.25	0.5	1	2	4	8	16	32	64	>64	Total
Citrobacter freundii complex	191	34	9	3								237
Citrobacter koseri	90											90
Enterobacter cloacae	64	23	19	13	1							120
Escherichia coli	89	13	9	2	1		3	3				120
Klebsiella aerogenes	73	6	5	3	3							90
Klebsiella pneumoniae	76	13	11	5	12	3						120
Morganella morganii	88			1			1					90
Proteus mirabilis	111	6	2									119
Proteus vulgaris	83	2			2	3						90
Providencia species	80	5	5									90
Pseudomonas aeruginosa		3	2	28	46	14	17	10				120
Serratia marcescens	64	18	3	2				1	2			90
Total	1009	123	65	57	65	20	21	14	2			1376

CONCLUSION

The development data showed that cefepime-taniborbactam MIC results for Enterobacterales and *P. aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels. Essential agreement is >90% for all inoculation and read methods. This development data supports the continued evaluation of MSDGN panel with cefepime-taniborbactam in a multicenter trial.

References

1. Hamrick et al., 2020, “VNRX-5133 (Taniborbactam), a Broad-Spectrum Inhibitor of Serine- and Metallo-β-Lactamases, Restores Activity of Cefepime in Enterobacterales and *Pseudomonas aeruginosa*”, ASM Journals, Antimicrobial Agents and Chemotherapy, Vol. 64, No. 3, https://doi.org/10.1128/AAC.01963-19.
2. Liu et al., 2020, “Discovery of Taniborbactam (VNRX-5133): A Broad-Spectrum Serine- and Metallo-β-lactamase Inhibitor for Carbapenem-Resistant Bacterial Infections)”, Journal of Medicinal Chemistry 2020 63 (6), 2789-2801, DOI: 10.1021/acs.jmedchem.9b01518.
3. Venatorx Pharmaceuticals, Inc. (2019, August 7 – 2021, December 14) “Safety and Efficacy Study of Cefepime/VNRX-5133 in Patients With Complicated Urinary Tract Infections (CERTAIN-1)”, NCT03840148.
4. Clinical and Laboratory Standards Institute. 2018. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 11th ed CLSI supplement M07 Clinical and Laboratory Standards Institute, Wayne, PA.
5. Clinical and Laboratory Standards Institute. 2022. Performance standards for antimicrobial susceptibility testing. 32nd ed CLSI supplement M100 Clinical and Laboratory Standards Institute, Wayne, PA.

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