Multicenter Evaluation of Meropenem/Vaborbactam MIC Results for *Enterobacterales and Pseudomonas aeruginosa* Using EUCAST breakpoints on MicroScan Dried Gram Negative MIC Panels

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ABSTRACT

Background: A multicenter study was performed to evaluate the accuracy of testing meropenem/vaborbactam on a MicroScan Dried Gram-negative MIC (MSDGN) Panel when compared to a frozen ISO/CLSI broth microdilution reference panel.

Materials/Methods: An evaluation was conducted at three U.S. sites by comparing MIC values obtained using the MSDGN to MICs utilizing an ISO/CLSI broth microdilution reference panel. A total of 775 *Enterobacterales* and *P. aeruginosa* clinical isolates were tested using the Prompt*[®] and turbidity methods of inoculation during the efficacy phase. A subset of 14 organisms for reproducibility was tested on MSDGN panels at each site. MSDGN panels were incubated at 35 ± 1°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually. Read times for the MSDGN panels were at 16-20 hours. Frozen reference panels were prepared and incubated according to ISO/CLSI methodology and read visually. EUCAST breakpoints (mg/L) used for interpretation of MIC results were: *Enterobacterales* ≤ 8/8 S, > 8/8 R and *P. aeruginosa* ≤ 8/8 S, > 8/8 R.

Results: Essential and categorical agreement when compared to frozen reference panel results, for all isolates tested in efficacy as follows:

Read Method	Essential Agreement %		Categorical Agreement %		Very Major Errors (VMJ)* %		Major Errors (MAJ)* %	
	P	T	P	T	P	T	P	T
WalkAway	99.2 (769/775)	99.2 (769/775)	99.5 (771/775)	99.5 (771/775)	0.0 (0/6)	0.0 (0/6)	0.1 (1/769)	0.3 (2/769)
autoSCAN-4	99.2 (769/775)	99.1 (768/775)	99.7 (773/775)	99.5 (771/775)	0.0 (0/6)	0.0 (0/6)	0.1 (1/769)	0.3 (2/769)
Visually	99.5 (771/775)	99.1 (768/775)	99.9 (774/775)	99.6 (772/775)	0.0 (0/6)	0.0 (0/6)	0.1 (1/769)	0.3 (2/769)

P = Prompt inoculation method, T = Turbidity inoculation method
*Calculation of MAJ and VMJ excluding 1 well errors

Reproducibility among the three sites were greater than 95% for all read methods for both the Prompt and turbidity inoculation methods.

Conclusion: This multicenter study showed that meropenem/vaborbactam MIC results for *Enterobacterales* and *P. aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using EUCAST interpretive criteria.

INTRODUCTION

A multicenter study was performed to evaluate the performance of meropenem/vaborbactam with EUCAST criteria on a MicroScan Dried Gram Negative MIC panel using *Enterobacterales* and *P. aeruginosa* isolates.

METHODS

Study Design: MicroScan Dried Gram Negative MIC panels were tested concurrently with an ISO/CLSI frozen broth microdilution reference panel at three sites using both the Prompt and turbidity Inoculation methods.

A total of 775 *Enterobacterales* and *P. aeruginosa* clinical isolates were tested among the three sites.

Quality Control Expected Results, CLSI M100-ED30*
Escherichia coli ATCC 25922: ≤0.03/8 – 0.06/8 µg/mL
Pseudomonas aeruginosa ATCC 27853: 0.12/8 – 1/8 µg/mL
Escherichia coli ATCC 35218 : ≤0.03/8 – 0.06/8 µg/mL
Klebsiella pneumoniae ATCC 700603: ≤0.03/8 – 0.06/8 µg/mL
Klebsiella pneumoniae ATCC BAA-1705: ≤0.03/8 – 0.06/8 µg/mL
Note: ranges listed above are extrapolated to the panel dilutions

Panels
Frozen reference and MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of meropenem/vaborbactam 0.03/8 – 64/8 mg/L in cation-adjusted Mueller-Hinton broth.
Frozen reference panels were prepared and inoculated following ISO/CLSI recommendations.

Reproducibility
Organisms with on-scale results for meropenem/vaborbactam were tested in triplicate (for each inoculation method) on the MicroScan Dried Gram Negative MIC panels and singly on the frozen reference panel on three different days at each site.
MicroScan Dried Gram Negative MIC panels were tested using both the Prompt and turbidity inoculation methods and read on the WalkAway system, autoSCAN-4 instrument and manually.
The mode was calculated for each isolate and reproducibility was determined as within ± 1 well of the two-fold dilution of the calculated mode for each isolate.

Quality Control
Quality control (QC) testing was performed daily using ATCC 25922 *E. coli*, ATCC 27853 *P. aeruginosa*, ATCC 35218 *E. coli*, ATCC 700603 *K. pneumoniae*, ATCC BAA-1705 *K. pneumoniae* using extrapolated CLSI M100-ED30 QC ranges based on the panel dilutions.

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 34-37°C 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±1°C in the WalkAway system for 18±2 hours. All panels were read by the WalkAway, autoSCAN-4, and visually.

DATA ANALYSIS AND RESULTS

Data Analysis
Essential Agreement (EA) = MSDGN panel MIC within ± 1 well of the two-fold dilution of the frozen reference result MIC.
Categorical Agreement (CA) = MSDGN panel and reference categorical results (S, R) agree using EUCAST breakpoints for *Enterobacterales and P. aeruginosa*. (Table 1).

Table 1. Meropenem/Vaborbactam (EUCAST v10.0) Interpretive Breakpoints (mg/L)

Organism Group	Susceptible	Resistant
Enterobacterales	≤ 8/8	> 8/8
P. aeruginosa	≤ 8/8	> 8/8

Major Errors = Frozen reference MIC is S and MSDGN panel MIC is R; calculated for susceptible strains only.

$$\% \text{ Major Errors} = \frac{\text{No. Major Errors}}{\text{Total No. S Isolates tested}} \times 100$$

Very Major Errors = Frozen reference is R and MSDGN panel MIC is S; calculated for resistant strains only.

$$\% \text{ Very Major Errors} = \frac{\text{No. Very Major Errors}}{\text{Total No. R Isolates tested}} \times 100$$

Quality Control
Overall QC results for the MSDGN panel were 94.9-100% in range for organisms tested. Overall QC results for frozen reference panel were 99.2–100% in range for organism tested.

Reproducibility (Table 1)
Overall agreement (within ± two-fold dilution) between all sites for the reproducibility phase was ≥ 95% for all combinations.

Table 4. Reproducibility Testing—All Sites Combined with Manual, WalkAway, and autoScan-4 Instrument Reads

Read Method	Inoculation Method	No. (%) Agreement All Sites Combined
WalkAway	Prompt	363/378 (96.0)
autoSCAN-4	Prompt	362/378 (95.8)
Manual	Prompt	366/378 (96.8)
WalkAway	Turbidity	371/378 (98.1)
autoSCAN-4	Turbidity	371/378 (98.1)
Manual	Turbidity	367/378 (97.1)

Efficacy - Prompt (Table 2)
Essential Agreement for *Enterobacterales* and *P. aeruginosa* between MSDGN panel and frozen reference panel was 99.2% (769/775) for WalkAway System method, 99.2% (769/775) for manual read method using the Prompt inoculation method.
Categorical Agreement for *Enterobacterales* and *P. aeruginosa* between MSDGN panel and frozen reference panel was 99.5% (771/775) for WalkAway System method, 99.7% (773/775) for autoSCAN-4 instrument, 99.9% (774/775) for manual read method using the Prompt inoculation method.

Table 2. Clinical Isolates - Prompt Inoculation Method

Read Method	Essential Agreement		Categorical Agreement		Major Errors*		Very Major Errors*	
	No.	%	No.	%	No.	%	No.	%
WalkAway	769/775	99.2	771/775	99.5	1/769	0.1	0/6	0.0
autoSCAN-4	769/775	99.2	773/775	99.7	1/769	0.1	0/6	0.0
Visually	771/775	99.5	774/775	99.9	1/769	0.1	0/6	0.0

*Calculation of MAJ and VMJ excluding 1 well errors

Efficacy - Turbidity (Table 3)
Essential Agreement for *Enterobacterales* and *P. aeruginosa* between MSDGN panel and frozen reference panel was 99.2% (769/775) for WalkAway System method, 99.1% (768/775) for autoSCAN-4 instrument, 99.1% (768/775) for manual read method using the turbidity inoculation method.
Categorical Agreement for *Enterobacterales* and *P. aeruginosa* between MSDGN panel and frozen reference panel was 99.5% (771/775) for WalkAway System method, 99.5% (771/775) for autoSCAN-4 instrument, 99.6% (772/775) for manual read method using the turbidity inoculation method.

Table 3. Clinical Isolates – Turbidity Inoculation Method

Read Method	Essential Agreement		Categorical Agreement		Major Errors*		Very Major Errors*	
	No.	%	No.	%	No.	%	No.	%
WalkAway	769/775	99.2	771/775	99.5	2/769	0.3	0/6	0.0
autoSCAN-4	768/775	99.1	771/775	99.5	2/769	0.3	0/6	0.0
Visually	768/775	99.1	772/775	99.6	2/769	0.3	0/6	0.0

*Calculation of MAJ and VMJ excluding 1 well errors

Meropenem/vaborbactam MIC values tended to be one or more doubling dilution lower when compared to the reference broth microdilution method for *P. aeruginosa* with the autoSCAN-4 and manual reads when using the turbidity Inoculation system

CONCLUSION

This multicenter study showed that meropenem/vaborbactam MIC results for *Enterobacterales and P. aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using EUCAST interpretive criteria.