Development of Meropenem-Vaborbactam MIC Assay for Gram Negative Bacteria on MicroScan Dried Gram Negative MIC Panels

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ABSTRACT

Background: Development of a meropenem-vaborbactam assay was completed for the MicroScan Dried Gram Negative (MSDGN) MIC Panel when compared to CLSI broth microdilution reference panels.
Material/methods: Development was conducted by comparing MICs obtained using the MSDGN panel to MICs using a CLSI broth microdilution reference panel. A total of 197 *Enterobacteriaceae*, and 31 *Pseudomonas aeruginosa* isolates were tested (for a total of 228 isolates) using the 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. Read times for the MSDGN panels were at 16-20 hours for the autoSCAN-4 instrument and visual reads, and 16-18 hours for the WalkAway System. Each isolate was read in triplicate on the autoSCAN-4 instrument. 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. Frozen reference panels were read at 16-20 hours. FDA breakpoints (µg/ml) used for interpretation of MIC results were: *Enterobacteriaceae* ≤ 4/8 S, 8/8 I, and ≥ 16/8 R.
Results: When compared to frozen reference panel results, essential agreement for all isolates testing and categorical agreement for all isolates tested are as follows:

Read Method	Essential Agreement %		Categorical Agreement %	
	T	P	T	P
Visual	95.6 (218/228)	92.1 (209/227)	98.5 (194/197)	97.9 (193/197)
WalkAway	93.8 (165/176)	90.3 (158/175)	97.4 (147/151)	97.4 (147/151)
autoSCAN-4	94.0 (643/684)	90.9 (619/681)	97.9 (579/591)	97.9 (579/591)

T = Turbidity inoculation method, P = Prompt* inoculation method

Read Method	VMJ %		MAJ %		MIN %	
	T	P	T	P	T	P
Visual	16.7 (1/6)	0.0 (0/6)	0.0 (0/187)	0.0 (0/187)	1.0 (2/197)	2.0 (4/197)
WalkAway	20.0 (1/5)	20.0 (1/5)	0.0 (0/142)	0.0 (0/142)	1.9 (3/151)	1.9 (3/151)
autoSCAN-4	27.8 (5/18)	16.7 (3/18)	0.0 (0/561)	0.0 (0/561)	1.2 (7/591)	1.5 (9/591)

T = Turbidity inoculation method, P = Prompt* inoculation method

Conclusion: The MIC results obtained with the MSDGN panel with an extended dilution series correlate well with MICs obtained using frozen reference panels. The elevated Very Major Error Rate may be inflated due to the low number of resistant isolates tested.

INTRODUCTION

MicroScan Dried Gram Negative (MSDGN) MIC panels were developed for testing of Gram Negative bacteria with meropenem-vaborbactam (MEV) and FDA interpretive breakpoints.

METHODS

Study Design
MSDGN MIC panels were tested concurrently with a CLSI frozen broth microdilution reference panel using both the turbidity and Prompt®* Inoculation methods.
A total of 192 *Enterobacteriaceae* and 31 *Pseudomonas aeruginosa* isolates were tested.

METHODS (Continued)

Panels
• Frozen reference and MSDGN MIC panels contained two-fold doubling dilutions of meropenem-vaborbactam 0.004-64µg/ml in cation-adjusted Mueller-Hinton broth.
• Reference panels were prepared and frozen following CLSI/ISO recommendations.
Quality Control
• Quality control (QC) testing was performed daily using ATCC organisms and the FDA ranges listed in Table 1.

Table 1. Quality Control Expected Results (µg/ml)

Escherichia coli ATCC 25922	0.008/8-0.06/8
Escherichia coli ATCC 35218	0.008/8-0.06/8
Klebsiella pneumoniae ATCC 700603	0.015/8-0.06/8
Klebsiella pneumoniae ATCC BAA-1705	0.015/8-0.06/8
Pseudomonas aeruginosa ATCC 27853	0.12/8-1/8

Panel Inoculation, Incubation, and Reading
• All isolates were subcultured into trypticase soy agar (TSA) with 5% sheep blood and incubated for 18-24 hours at 35-37ºC 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 also incubated at 35±2ºC.
• MSDGN MIC panels were read at 16-20 hours for the autoSCAN-4 instrument and visual reads, and 16-18 hours in the WalkAway system.
• Each isolate was read in triplicate on the autoSCAN-4 instrument.
• Frozen reference panels were incubated at 35±1ºC in the incubator for 16-20 hours. All panels were read visually.
Data Analysis
•Essential Agreement (EA) = MSDGN panel MIC within +/- 1 dilution of the frozen reference result MIC.
•Categorical Agreement (CA) = MSDGN panel and reference categorical results (S, I, R) agree using FDA breakpoints. (Table 2).

Table 2. Meropenem-Vaborbactam FDA Interpretive Breakpoints (µg/ml)**

Organism Group	S	I	R
Enterobacteriaceae	≤4/8	8/8	≥16/8

• Major Errors (MAJ) = 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 (VMJ) = Frozen reference is R and MSDGN panel MIC is S; calculated for resistant strains only.

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

• Minor Errors (MIN) = Frozen reference is S or R when MSGDN panel MIC is I or MSDGP panel MIC is S or R when frozen reference is I; calculated for all isolates tested.

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

RESULTS

Efficacy (Tables 3 - 6)
A total of 228 isolates were tested using the turbidity and Prompt®* inoculation methods by a visual read, on the WalkAway System, and the autoSCAN-4 instrument.

Table 3. *Enterobacteriaceae* – Turbidity Inoculation Method

	Essential Agreement		Categorical Agreement		Minor Errors		Major Errors		Very Major Errors	
Read Method	No.	%	No.	%	No.	%	No.	%	No.	%
Visual	189/197	95.9	194/197	98.5	2/197	1.0	0/187	0.0	1/6	16.7
WalkAway	142/151	94.0	147/151	97.4	3/151	1.9	0/142	0.0	1/5	20.0
autoSCAN-4	559/591	94.6	579/591	97.9	7/591	1.2	0/561	0.0	5/18	27.8

Table 4. *Enterobacteriaceae* – Prompt®* Inoculation Method

	Essential Agreement		Categorical Agreement		Minor Errors		Major Errors		Very Major Errors	
Read Method	No.	%	No.	%	No.	%	No.	%	No.	%
Visual	183/197	92.9	193/197	97.9	4/197	2.0	0/187	0.0	0/6	0.0
WalkAway	136/151	90.1	147/151	97.4	3/151	1.9	0/142	0.0	1/5	20.0
autoSCAN-4	541/591	91.5	579/591	97.9	9/591	1.5	0/561	0.0	3/18	16.6

Table 5. *Pseudomonas aeruginosa* – Turbidity Inoculation Method

	Essential Agreement	
Read Method	No.	%
Visual	29/31	93.6
WalkAway	23/25	92.0
autoSCAN-4	84/93	90.3

Table 6. *Pseudomonas aeruginosa* – Prompt®* Inoculation Method

	Essential Agreement	
Read Method	No.	%
Visual	26/30	86.7
WalkAway	22/24	91.7
autoSCAN-4	78/90	86.7

Quality Control (Table 7)
Overall >95% of QC results were in range.

Table 7. Quality Control Results (visual, WalkAway and autoSCAN-4 read methods)

Organism	QC Range (µg/mL)	Ref	Percent (%) in Range					
			Visual		WalkAway		autoSCAN-4	
			Turbidity	Prompt	Turbidity	Prompt	Turbidity	Prompt
***E.coli* ATCC 25922**	0.008/8-0.06/6	100	100	100	100	100	100	100
***E.coli* ATCC 35218**	0.008/8-0.06/6	100	100	100	100	100	100	100
***P. aeruginosa* ATCC 27853**	0.12/8-1/8	100	100	100	98.2	100	100	98.9
***K. pneumoniae* ATCC 700603**	0.015/8-0.06/8	98.0	96.2	100	96.5	100	96.6	100
***K. pneumoniae* ATCC BAA-1705**	0.015/8-0.06/8	97.8	97.8	100	98.1	100	99.0	100

CONCLUSION

Acceptable correlation (EA >90%) for all isolates combined was observed between MICs obtained using MicroScan Dried Gram-Negative panel and MICs obtained using a CLSI broth microdilution frozen reference panel for susceptibility testing of meropenem-vaborbactam with *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Acceptable categorical agreement (CA >90%) was observed for *Enterobacteriaceae*. The observed Very Major Error rate may be inflated due to the low number of resistant isolates tested.

* PROMPT® is a registered trademark of 3M.
** *Pseudomonas aeruginosa* is included in the “in vitro” section of the FDA label, however there are no FDA Interpretive Breakpoints for *Pseudomonas aeruginosa* with meropenem-vaborbactam.