

# Multicenter Evaluation of Imipenem MIC Results at 18 hours for Gram-Negative Organisms Using MicroScan Dried Gram Negative MIC Panels

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## INTRODUCTION

A multicenter study was performed to evaluate the performance for MicroScan Dried Gram-Negative (MSDGN) MIC panels with a revised formulation of imipenem using gram-negative efficacy isolates which included various members of the Enterobacteriaceae group, *Acinetobacter* spp., and *Pseudomonas aeruginosa*.

## METHODS

### Study Design

### Clinical Isolate Testing

•MSDGN panels were tested concurrently with a CLSI frozen broth microdilution reference panel at four sites using both the turbidity and Prompt™ Inoculation methods.

•A total of 419 clinical efficacy isolates:

- 39 *Acinetobacter* spp.
- 295 Enterobacteriaceae including specific isolates with known mechanisms of resistance
- 85 *Pseudomonas aeruginosa*

### Panels

•Frozen reference and MSDGN panels contained two-fold doubling dilutions of imipenem 0.25-32 µg/mL in cation-adjusted Mueller-Hinton broth.

•Reference panels were prepared and frozen following CLSI/ISO recommendations.

### Reproducibility

•Reproducibility organisms with known results on-scale for Imipenem were tested in triplicate (for each inoculation method) on the MSDGN panels and singly on the frozen reference panel on three different days at each site.

•MSDGN panels were tested using both the turbidity and Prompt inoculation methods and read manually, on the WalkAway system, and the autoSCAN-4 instrument.

### Quality Control

•Quality control (QC) testing was performed daily using ATCC 25922 *E. coli* and ATCC 27853 *P. aeruginosa* using FDA QC ranges.

### Quality Control Expected Results

- E. coli* ATCC 25922: ≤0.25 µg/mL
- P. aeruginosa* ATCC 27853: 1-4 µg/mL

### Panel Inoculation, Incubation, and Reading

•All isolates were subcultured in trypticase soy agar (TSA) with 5% sheep blood and incubated for 18-24 hours at 35-37°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 panels and frozen reference panels. MSDGN panels were also inoculated using the Prompt Inoculation method.

•Following inoculation, MSDGN panels were also incubated at 35±2°C in WalkAway system. All panels were read manually, on the WalkAway system, and the autoSCAN-4 instrument.

•Frozen reference panels were read and reported at 16-20 hours for all organisms except *Acinetobacter* spp., which were read at 20-24 hours.

•Read times for the MSDGN panels were at 18 hours for all species

## METHODS (Continued)

### 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 1).

Table 1 Imipenem FDA Interpretive Breakpoints (µg/mL)

Organism Group	Imipenem FDA Breakpoints		
	S	I	R
Enterobacteriaceae	≤ 1	2	≥ 4
Acinetobacter spp.	≤ 2	4	≥ 8
Pseudomonas aeruginosa	≤ 2	4	≥ 8

•Major Errors = Frozen reference is S and MSDGN panel 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 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 = Frozen reference is S or R when MSDGN panel is I or MSDGN panel 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 2,3,4,5,6 and 7)

•A total of 419 clinical efficacy isolates were tested among three sites using the turbidity and Prompt inoculation method by manual read, on the WalkAway System and the autoSCAN-4 instrument. Performance data presented is based on mapped dilution range of 0.25 – 8 µg/mL.

Table 2. Efficacy – WalkAway Read, turbidity Inoculation Method

Manual Read	Essential Agreement		Categorical Agreement		Minor Errors		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%	No.	%
Organism Group										
Acinetobacter spp.	39/39	100	36/39	92.3	3/39	7.7	0/19	0.0	0/17	0.0
Enterobacteriaceae <sup>^</sup>	294/295	99.7	281/295	95.3	14/295	4.7	0/245	0.0	0/31	0.0
P. aeruginosa	81/85	95.3	79/85	92.9	5/85	5.9	1/68	1.5	0/13	0.0
Overall	410/419	97.9	384/419	91.6	32/419	7.6	3/332	0.9	0/61	0.0

Table 3. Efficacy – WalkAway Read, Prompt Inoculation Method

Manual Read	Essential Agreement		Categorical Agreement		Minor Errors		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%	No.	%
Organism Group										
Acinetobacter spp.	37/39	94.9	35/39	89.7	3/39	7.7	1/19	5.3	0/17	0.0
Enterobacteriaceae <sup>^</sup>	285/295	96.6	272/295	92.2	23/295	7.8	0/245	0.0	0/31	0.0
P. aeruginosa	82/85	96.5	80/85	94.1	4/85	4.7	1/68	1.5	0/13	0.0
Overall	404/419	96.4	387/419	92.4	30/419	7.2	2/332	0.6	0/61	0.0

<sup>^</sup> - Enterobacteriaceae include 21 Citrobacter species, 87 Escherichia coli, 60 Enterobacter species, 86 Klebsiella species, 16 Morganella morganii, 11 Providencia rettgeri, and 14 Proteus species excluding Proteus mirabilis

## RESULTS (Continued)

Table 4. Efficacy – Manual Read, turbidity Inoculation Method

Manual Read	Essential Agreement		Categorical Agreement		Minor Errors		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%	No.	%
Organism Group										
Acinetobacter spp.	39/39	100	36/39	92.3	3/39	7.7	0/19	0.0	0/17	0.0
Enterobacteriaceae <sup>^</sup>	290/295	98.3	269/295	91.2	24/295	8.1	2/245	0.8	0/31	0.0
P. aeruginosa	83/85	97.6	82/85	96.5	2/85	2.4	1/68	1.5	0/13	0.0
Overall	412/419	98.3	387/419	92.4	29/419	6.9	3/332	0.9	0/61	0.0

Table 6. Efficacy – autoSCAN-4 Read, turbidity Inoculation Method

Manual Read	Essential Agreement		Categorical Agreement		Minor Errors		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%	No.	%
Organism Group										
Acinetobacter spp.	39/39	100	36/39	92.3	3/39	7.7	0/19	0.0	0/17	0.0
Enterobacteriaceae <sup>^</sup>	294/295	99.7	290/295	98.3	5/295	1.7	0/245	0.0	0/31	0.0
P. aeruginosa	83/85	97.6	82/85	96.5	2/85	2.4	1/68	1.5	0/13	0.0
Overall	412/419	98.3	390/419	93.1	26/419	6.2	3/332	0.9	0/61	0.0

Note: Final cleared performance claims for the WalkAway read method and the Prompt inoculation method for efficacy and challenge isolates combined (n=598) were 96.8% (579/598) essential agreement and 92.0% (550/598) categorical agreement.

### Quality Control

Overall QC results for the MSDGN panel were demonstrated ≥ 95% in range for both *E. coli* and *P. aeruginosa* using both inoculation methods.

Table 8 – Quality Control

Organism	QC Range (µg/mL)	Ref	Percent (%) in Range						Number and Distribution of Results							
			Manual		WalkAway		autoSCAN-4		MIC (µg/mL)	Ref	Manual		WalkAway		autoSCAN-4	
			Turbidity	Prompt	Turbidity	Prompt	Turbidity	Prompt			Turbidity	Prompt	Turbidity	Prompt		
<i>E. coli</i> ATCC 25922	≤0.25	98.9	96.8	96.5	95.7	95.0	96.8	96.5	0.25	187	183	193	182	190	182	193
	0.5	1	3	4	5	8	3	5	0.5	1	3	4	5	8	3	5
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1
	4	1	1	1	1	1	1	1	4	1	1	1	1	1	1	1
	8								8							
	16								16							
	32								32							
<i>P. aeruginosa</i> ATCC 27853	1-4	100	99.4	100	98.9	98.5	99.4	100	0.25							
	0.5								0.5							
	1	32	43	22	5	1	20	13	1	32	43	22	5	1	20	13
	2	149	142	175	177	185	164	181	2	149	142	175	177	185	164	181
	4	8	1	4	2	13		6	4	8	1	4	2	13		6
	8		1	2	3	1			8		1	2	3	1		
	16								16							
	32								32							
>32								>32								

### Reproducibility

Overall agreement (within ± two fold dilution) between all sites for the reproducibility phase was ≥ 95% for all combinations when compared to a frozen reference panel tested the same day (expected value.)

Table 9. Reproducibility

Read Method	Inoculation Method	No. (%) Agreement All Sites Combined
Manual	Turbidity	270/270 (100)
WalkAway		267/270 (98.9)
autoSCAN-4		266/270 (98.5)
Manual	Prompt™	268/270 (99.3)
WalkAway		267/270 (98.9)
autoSCAN-4		269/270 (99.6)

## CONCLUSION

This multicenter study showed that the improved imipenem MIC results for Gram negative bacilli obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels.

† Deceased 29 November 2016

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