

# Multicenter Evaluation of Cefazolin MIC Results for *Enterobacterales* Using CLSI Breakpoints on MicroScan Dried Gram Negative MIC Panels

C. Emery<sup>1</sup>, A. Harrington<sup>2</sup>, S. DesJarlais<sup>2</sup>, O.B. Garner<sup>3</sup>, C.J. Hastey<sup>4</sup>, R.K. Brookman<sup>4</sup>, Z.C. Lockett<sup>4</sup>, and J.Y. Chau<sup>4</sup>

<sup>1</sup>Indiana University School of Medicine, Indianapolis, IN, <sup>2</sup>Loyola University Medical Center, Maywood, IL, <sup>3</sup>UCLA David Geffen School of Medicine, Los Angeles, CA, and <sup>4</sup>Beckman Coulter, West Sacramento, CA

## ABSTRACT

**Background:** A multicenter study was performed to evaluate the accuracy of cefazolin on MicroScan Dried Gram-negative MIC (MSDGN) Panels when compared to frozen CLSI/ISO broth microdilution reference panels.

**Materials/Methods:** A total of 450 *Enterobacterales* clinical isolates were tested using the turbidity and Prompt® methods of inoculation during the efficacy phase at three U.S. sites. An evaluation was conducted by comparing MIC values obtained using the MSDGN panels to MICs utilizing a CLSI/ISO broth microdilution reference panel. A subset of 12 organisms was tested on MSDGN panels at each site during reproducibility. MSDGN panels were incubated at 35 ± 1°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually at 18 hours. Frozen reference panels were prepared according to CLSI/ISO methodology, incubated for 16-20 hours and read visually. CLSI breakpoints (µg/mL) used for interpretation of MIC results were: *Enterobacterales* ≤ 2 S, 4 I, ≥ 8 R.

**Results:** Reproducibility among the three sites were greater than 95% for all read methods for both the turbidity and Prompt inoculation methods. Essential, categorical agreement and categorical errors were calculated compared to MIC results from frozen reference panels for all isolates tested in efficacy and found in the following table.

Read Method	Essential Agreement %		Categorical Agreement %		Very Major Errors %		Major Errors %		Minor Errors%	
	P	T	P	T	P	T	P	T	P	T
WalkAway	97.1 (437/450)	97.1 (437/450)	97.6 (439/450)	98.0 (441/450)	1.6 (4/254)	1.2 (3/254)	3.4 (4/119)	0.0 (0/119)	0.6 (3/450)	1.3 (6/450)
autoSCAN-4	97.1 (437/450)	97.1 (437/450)	97.6 (439/450)	98.0 (441/450)	1.6 (4/254)	1.2 (3/254)	3.4 (4/119)	0.0 (0/119)	0.6 (3/450)	1.3 (6/450)
Manual	96.9 (436/450)	97.1 (437/450)	97.6 (439/450)	98.2 (442/450)	1.6 (4/254)	0.8 (2/254)	3.4 (4/119)	0.0 (0/119)	0.6 (3/450)	1.3 (6/450)
P = Prompt inoculation method, T = Turbidity inoculation method										
*Calculation of CA and MIN excluding 1 well errors										

**Conclusion:** This multicenter study showed that cefazolin MIC results for *Enterobacterales* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using CLSI interpretive criteria.

## INTRODUCTION

Data from a multicenter study was evaluated the performance of a MicroScan Dried Gram Negative MIC panel with cefazolin using *Enterobacterales* isolates with CLSI interpretive breakpoints.

## METHODS

**Study Design:** MicroScan Dried Gram Negative MIC panels were tested concurrently with a CLSI frozen broth microdilution reference panel at three sites using both the turbidity and Prompt Inoculation methods. A total of 450 *Enterobacterales* clinical isolates were tested among the three sites.

**Quality Control Expected Results**  
*Escherichia coli* ATCC 25922: 1 – 4 µg/ml, extrapolated to ≤ 1 – 4 on the test panel (CLSI M100-ED30)  
*Pseudomonas aeruginosa* ATCC 27853: > 16 µg/ml (MicroScan range)

## METHODS (Continued)

**Panels**  
Frozen reference and MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of cefazolin 1 - 32 µg/ml (0.5 - 32 µg/ml on the frozen reference panel) 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 25922 *E. coli* and ATCC 27853 *P. aeruginosa*.

**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 hours. All panels were read by the WalkAway, autoSCAN-4, and visually.

**Reproducibility**  
Reproducibility organisms with known results on-scale for cefazolin 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 turbidity and Prompt inoculation methods and read on the WalkAway system, autoSCAN-4 instrument and manually.

**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 CLSI breakpoints for *Enterobacterales*. (Table 1).

**Table 1. Cefazolin CLSI Interpretive Breakpoints (µg/ml) (CLSI M100-ED30)**

Organism Group	Susceptible (S)	Intermediate (I)	Resistant (R)
<i>Enterobacterales</i>	≤ 2	4	≥ 8

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

% Major Errors =

No. Major Errors

Total No. S Isolates tested

X 100

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

% Very Major Errors =

No. Very Major Errors

Total No. R Isolates tested

X 100

Minor Errors = Frozen reference MIC is S or R when MSGDN panel MIC is I or MSDGN panel MIC is S or R when frozen reference MIC is I; calculated for all isolates tested.

% Minor Errors =

No. Minor Errors

Total No. Isolates tested

X 100

## RESULTS

**Efficacy** (Tables 2 and 3)  
A total of 450 *Enterobacterales* clinical isolates were tested among three sites.

**Efficacy - Prompt**  
Essential Agreement for *Enterobacterales* between MSDGN panel and frozen reference panel was 97.1% (437/450) for WalkAway System method, 97.1% (437/450) for autoSCAN-4 instrument, 96.9% (436/450) for manual read method using the Prompt inoculation method.

Categorical Agreement for *Enterobacterales* between MSDGN panel and frozen reference panel was 97.6% (439/450) for WalkAway System method, 97.6% (439/450) for autoSCAN-4 instrument, 97.6% (439/450) for manual read method using the Prompt inoculation method.

**Table 2. Clinical Isolates - Prompt Inoculation Method**

Read Method	Essential Agreement		Categorical Agreement*		Minor Errors*		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%	No.	%
WalkAway	437/450	97.1	439/450	97.6	3/450	0.6	4/119	3.4	4/254	1.6
autoSCAN-4	437/450	97.1	439/450	97.6	3/450	0.6	4/119	3.4	4/254	1.6
Manual	436/450	96.9	439/450	97.6	3/450	0.6	4/119	3.4	4/254	1.6
*Calculation of CA and MIN excluding 1 well errors										

**Efficacy - Turbidity**  
Essential Agreement for *Enterobacterales* between MSDGN panel and frozen reference panel was 97.1% (437/450) for WalkAway System method, 97.1% (437/450) for autoSCAN-4 instrument, 97.1% (437/450) for manual read method using the turbidity inoculation method.

Categorical Agreement for *Enterobacterales* between MSDGN panel and frozen reference panel was 98.0% (441/450) for WalkAway System method, 98.0% (441/450) for autoSCAN-4 instrument, 98.2% (442/450) for manual read method using the turbidity inoculation method.

**Table 3. Clinical Isolates – Turbidity Inoculation Method**

Read Method	Essential Agreement		Categorical Agreement*		Minor Errors*		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%	No.	%
WalkAway	437/450	97.1	441/450	98.0	6/450	1.3	0/119	0.0	3/254	1.2
autoSCAN-4	437/450	97.1	441/450	98.0	6/450	1.3	0/119	0.0	3/254	1.2
Manual	437/450	97.1	442/450	98.2	6/450	1.3	0/119	0.0	2/254	0.8
*Calculation of CA and MIN excluding 1 well errors										

**Reproducibility** (Table 4)  
A total of 12 isolates were tested for reproducibility at all three sites in triplicate over three days.

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

**Table 4. Reproducibility Testing with CFZ – All Sites Combined**

Read Method	Inoculation Method	No. (%) Agreement All Sites Combined
WalkAway	Prompt	324/324 (100)
autoSCAN-4		323/324 (99.7)
Manual		324/324 (100)
WalkAway	Turbidity	321/324 (99.1)
autoSCAN-4		322/324 (99.4)
Manual		321/324 (99.1)

**Quality Control** (Table 5)  
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 5. Quality Control**

Organism	QC Range (mg/L)	WalkAway		autoSCAN-4		Visually	
		Prompt	Turbidity	Prompt	Turbidity	Prompt	Turbidity
<i>E. coli</i> ATCC 25922	≤ 1 - 4	100%	100%	100%	100%	100%	100%
<i>P. aeruginosa</i> ATCC 27853	> 16	100%	100%	100%	100%	100%	100%

## CONCLUSION

This multicenter study showed that cefazolin MIC results for *Enterobacterales* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using CLSI interpretive criteria.

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