

Multicenter Evaluation of Amoxicillin/Clavulanic Acid MIC Results for *Enterobacteriaceae* Using MicroScan Dried Gram Negative MIC Panels

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ABSTRACT

Background: A multicenter study was performed to evaluate the accuracy of the EUCAST formulation of amoxicillin/clavulanic acid (2 µg/ml fixed clavulanic acid concentration) on a MicroScan Dried Gram Negative MIC (MSDGN) Panel when compared to frozen ISO/CLSI broth microdilution reference panels.

Material/methods: For efficacy, an evaluation was conducted at three sites by comparing MICs obtained using the MSDGN panel to MICs using an ISO/CLSI broth microdilution reference panel. A total of 626 *Enterobacteriaceae* clinical isolates were tested using the turbidity and Prompt[®] methods of inoculation. For reproducibility, a subset of 11 organisms was tested on MSDGN panels at all three sites. MSDGN panels were incubated at 35 ± 2°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually at 16-20 hours. Frozen reference panels, prepared according to ISO/CLSI methodology, were inoculated using the turbidity inoculation method. All frozen reference panels were incubated at 35 ± 2°C and read visually. EUCAST breakpoints (µg/ml) used for interpretation of MIC results were: *Enterobacteriaceae* ≤8/2 S and > 8/2 R.

Results: When compared to frozen reference panel results, essential and categorical agreements for Efficacy isolates are as follows:

Read Method	Essential Agreement %		Categorical Agreement %		Very Major Errors [^] %		Major Errors [^] %	
	T	P	T	P	T	P	T	P
Visually	96.8 (606/626)	94.6 (592/626)	96.0 (601/626)	95.5 (598/626)	1.0 (3/286)	0.3 (1/286)	0.9 (3/340)	1.8 (6/340)
WalkAway	96.3 (603/626)	95.7 (599/626)	95.7 (599/626)	95.2 (596/626)	0.7 (2/286)	0.3 (1/286)	1.5 (5/340)	2.1 (7/340)
autoSCAN-4	94.1 (589/626)	94.2 (590/626)	96.2 (602/626)	94.6 (592/626)	2.1 (6/286)	1.4 (4/286)	0.9 (3/340)	1.8 (6/340)

T = Turbidity inoculation method, P = Prompt inoculation method
[^] = calculated without 1 well dilution errors

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

Conclusions: This multicenter study showed that amoxicillin/clavulanic acid MIC results for *Enterobacteriaceae* obtained with the MSDGN panel with an extended dilution series correlate well with MICs obtained using frozen reference panels.

INTRODUCTION

A multicenter study was performed to evaluate the accuracy of a revised formulation of amoxicillin/clavulanic acid (2 µg/ml fixed clavulanic acid concentration) on a MicroScan Dried Gram Negative MIC (MSDGN) Panel when compared to frozen ISO/CLSI broth microdilution reference panels.

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 turbidity and Prompt Inoculation methods. A total of 626 *Enterobacteriaceae* clinical isolates were tested among the three sites.

Quality Control Expected Results (EUCAST v9.0)
Escherichia coli ATCC 25922: 2/2 - 8/2 µg/ml
Escherichia coli ATCC 35218: 4/2 - 32/2 µg/ml

METHODS (Continued)

Panels

•Frozen reference and MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of amoxicillin and fixed clavulanic acid (0.5/2 - 128/2 µg/ml) in cation-adjusted Mueller-Hinton broth. The 2 µg/mL fixed concentration of clavulanic acid follows EUCAST recommendations.

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

Reproducibility

•Reproducibility organisms with known results on-scale for amoxicillin/clavulanic acid 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.

Quality Control

•Quality control (QC) testing was performed daily using ATCC 25922 *E. coli* and ATCC 35218 *E. coli* using EUCAST, v9.0 QC ranges.

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 ± 2°C in the WalkAway system for 18 ± 2 hours. All panels were read by the WalkAway, autoSCAN-4, and 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, R) agree using EUCAST breakpoints for *Enterobacteriaceae*. (Table 1).

Table 1. Amoxicillin/Clavulanic Acid EUCAST v9.0 Interpretive Breakpoints (µg/ml)

Organism Group	S	R
<i>Enterobacteriaceae</i>	≤ 8/2	> 8/2

•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.

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

RESULTS

Efficacy (Tables 2 and 3)

Turbidity: A total of 626 *Enterobacteriaceae* clinical isolates were tested among three sites. MSDGN panels were inoculated using the turbidity inoculation method, Table 2.

•Essential Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 96.8% (606/626) for manual read method, 96.3% (603/626) for WalkAway System, 94.1% (589/626) for autoSCAN-4 instrument using the turbidity inoculation method.

•Categorical Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 96.0% (601/626) for manual read method, 95.7% (599/626) for WalkAway System, 96.2% (602/626) for autoSCAN-4 instrument using the turbidity inoculation method.

Table 2. Clinical Isolates - Turbidity Inoculation Method

Read Method	Essential Agreement		Categorical Agreement		Major Errors [^]		Very Major Errors [^]	
	No.	%	No.	%	No.	%	No.	%
Manual	606/626	96.8	601/626	96.0	3/340	0.9	3/286	1.0
WalkAway	603/626	96.3	599/626	95.7	5/340	1.5	2/286	0.7
autoSCAN-4	589/626	94.1	602/626	96.2	3/340	0.9	6/286	2.1

[^] = Errors that were within essential agreement were excluded from analysis due to variability acceptance of the test.

Prompt: A total of 626 *Enterobacteriaceae* clinical isolates were tested among three sites. MSDGN panels were inoculated using the Prompt inoculation method, Table 3.

•Essential Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 94.6% (592/626) for manual read method, 95.7% (599/626) for WalkAway System, 94.2% (590/626) for autoSCAN-4 instrument using the Prompt inoculation method.

•Categorical Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 95.5% (598/626) for manual read method, 95.2% (596/626) for WalkAway System, 94.6% (592/626) for autoScan-4 instrument using the Prompt inoculation method.

Table 3. Clinical Isolates – Prompt Inoculation Method

Read Method	Essential Agreement		Categorical Agreement		Major Errors [^]		Very Major Errors [^]	
	No.	%	No.	%	No.	%	No.	%
Manual	592/626	94.6	598/626	95.5	6/340	1.8	1/286	0.3
WalkAway	599/626	95.7	596/626	95.2	7/340	2.1	1/286	0.3
autoSCAN-4	590/626	94.2	592/626	94.6	6/340	1.8	4/286	1.4

[^] = Errors that were within essential agreement were excluded from analysis due to variability acceptance of the test.

Reproducibility (Tables 4 and 5)

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

Table 4. Reproducibility Testing with AUG – Best Case All Sites Combined with Manual, WalkAway, and autoSCAN-4 Instrument Reads of MicroScan Dried Gram-Negative Panel

Read Method	Inoculation Method	No. (%) Agreement Best Case All Sites Combined
Manual	Turbidity	296/297 (99.7)
WalkAway		293/297 (98.7)
autoSCAN-4		295/297 (99.3)
Manual	Prompt	292/297 (98.3)
WalkAway		294/297 (99.0)
autoSCAN-4		295/297 (99.3)

Table 5. Reproducibility Testing with AUG – Worst Case All Sites Combined with Manual, WalkAway, and autoSCAN-4 Instrument Reads of MicroScan Dried Gram-Negative Panel

Read Method	Inoculation Method	No. (%) Agreement Worst Case All Sites Combined
Manual	Turbidity	293/297 (98.7)
WalkAway		292/297 (98.3)
autoSCAN-4		286/297 (96.3)
Manual	Prompt	288/297 (97.0)
WalkAway		293/297 (98.7)
autoSCAN-4		283/297 (95.3)

Quality Control (Table 6)

•Overall QC results for the frozen reference panel were 100% (164/164) in range for ATCC 25922 *E. coli* and 99.3% (163/164) in range for ATCC 35218 *E. coli*.

Organism	QC Range (µg/mL)	Percent (%) in Range					
		Manual		WalkAway		autoSCAN-4	
		Turbidity	Prompt	Turbidity	Prompt	Turbidity	Prompt
<i>E. coli</i> ATCC 25922	2/2-8/2	164/164 100%	164/164 100%	162/162 100%	162/162 100%	164/164 100%	163/163 100%
<i>E. coli</i> ATCC 35218	4/2-32/2	162/164 98.7%	161/164 98.1%	162/164 98.7%	159/162 98.1%	162/164 98.7%	159/162 98.1%

CONCLUSION

This multicenter study showed that amoxicillin/clavulanic acid MIC results for *Enterobacteriaceae* obtained with the MSDGN panel with the EUCAST formulation correlate well with MICs obtained using frozen reference panels. The 2 µg/mL fixed concentration of clavulanic acid follows EUCAST recommendations.

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