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Multicenter Evaluation of Eravacycline MIC Results for *E. coli* Using MicroScan Dried Gram Negative MIC Panels

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ABSTRACT

Background: A multicenter study was performed to evaluate the accuracy of eravacycline on a MicroScan Dried Gram Negative MIC (MSDGN) Panel when compared to frozen 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 a CLSI broth microdilution reference panel. A total of 79 *E. coli* clinical isolates were tested using the turbidity and Prompt®* methods of inoculation. For reproducibility, a set of 11 organisms was tested on MSDGN panels at each site. 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. Frozen reference panels, prepared according to ISO/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 16-18 hours. EUCAST breakpoints (µg/ml) used for interpretation of MIC results were: *E. coli* ≤0.5 S and > 0.5 R.

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

Read Method	Essential Agreement %		Categorical Agreement %		Very Major Errors %		Major Errors %	
	T	P	T	P	T	P	T	P
Visually	100 (79/79)	97.5 (77/79)	100 (79/79)	98.7 (78/79)	0 (0/0)	0 (0/0)	0 (0/79)	1.3 (1/79)
WalkAway	98.7 (78/79)	94.9 (75/79)	100 (79/79)	98.7 (78/79)	0 (0/0)	0 (0/0)	0 (0/79)	1.3 (1/79)
autoSCAN-4	97.5 (77/79)	91.1 (72/79)	100 (79/79)	98.7 (78/79)	0 (0/0)	0 (0/0)	0 (0/79)	1.3 (1/79)

T = Turbidity inoculation method, P = Prompt inoculation method

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 eravacycline MIC results for *E. coli* 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 a MicroScan Dried Gram Negative MIC panel with eravacycline using *Escherichia coli* isolates with EUCAST 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 79 *E. coli* clinical isolates were tested among the three sites.

Quality Control Expected Results
Escherichia coli ATCC 25922: 0.03 – 0.12 µg/ml, EUCAST v9.0
Pseudomonas aeruginosa ATCC 27853: 2 - 16 µg/ml, CLSI M100-ED29

METHODS (Continued)

Panels
•Frozen reference and MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of eravacycline 0.016 - 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 eravacycline 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* using EUCAST QC range and ATCC 27853 *P. aeruginosa* using CLSI M100-ED29 QC range.

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 34-37ºC 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 incubated at 35±2ºC 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 *Enterobacterales, E. coli*. (Table 1).

Table 1. Eravacycline EUCAST v9.0 Interpretive Breakpoints (µg/ml)

Organism Group	Susceptible	Resistant
Enterobacterales, E. coli	≤ 0.5	> 0.5

•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)
•A total of 79 *Escherichia coli* clinical isolates were tested among three sites. MSDGN panels were inoculated using the turbidity inoculation method.
•Essential Agreement for *Escherichia coli* between MSDGN panel and frozen reference panel was 100% (79/79) for manual read method, 98.7% (78/79) for WalkAway System, 97.5% (77/79) for autoSCAN-4 instrument using the turbidity inoculation method.
•Categorical Agreement for *Escherichia coli* between MSDGN panel and frozen reference panel was 100% (79/79) for manual read method, 100% (79/79) for WalkAway System, 100% (79/79) for autoSCAN-4 instrument using the turbidity inoculation method.

Table 2. Clinical Isolates - Turbidity Inoculation Method

	Essential Agreement		Categorical Agreement		Major Errors		Very Major Errors	
Read Method	No.	%	No.	%	No.	%	No.	%
Manual	79/79	100	79/79	100	0/79	0	0/0	0
WalkAway	78/79	98.7	79/79	100	0/79	0	0/0	0
autoSCAN-4	77/79	97.5	79/79	100	0/79	0	0/0	0

•A total of 79 *Escherichia coli* clinical isolates were tested among three sites MSDGN panels were inoculated using the Prompt inoculation method.
•Essential Agreement for *Escherichia coli* between MSDGN panel and frozen reference panel was 97.5% (77/79) for manual read method, 94.9% (75/79) for WalkAway System, 91.1% (72/79) for autoSCAN-4 instrument using the Prompt inoculation method.
•Categorical Agreement for *Escherichia coli* between MSDGN panel and frozen reference panel was 98.7% (78/79) for manual read method, 98.7% (78/79) for WalkAway System, 98.7% (78/79) for autoScan-4 instrument using the Prompt inoculation method.

Table 3. Clinical Isolates – Prompt Inoculation Method

	Essential Agreement		Categorical Agreement		Major Errors		Very Major Errors	
Read Method	No.	%	No.	%	No.	%	No.	%
Manual	77/79	97.5	78/79	98.7	1/79	1.3	0/0	0
WalkAway	75/79	94.9	78/79	98.7	1/79	1.3	0/0	0
autoSCAN-4	72/79	91.1	78/79	98.7	1/79	1.3	0/0	0

Reproducibility (Table 4)
•Overall agreement (within ± one two-fold dilution) between all sites for the reproducibility phase was ≥ 95% for all combinations.

Table 4. Reproducibility Testing with ERV – 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	292/297 (98.3)
WalkAway		294/297 (99.0)
autoSCAN-4		293/297 (98.7)
Manual	Prompt	294/297 (99.0)
WalkAway		294/297 (99.0)
autoSCAN-4		294/297 (99.0)

Table 5. Reproducibility Worst Case

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

Quality Control (Table 6)
•Overall QC results for the frozen reference panel were 100% in range for ATCC 25922 *E. coli*, ATCC 27853 *P. aeruginosa*

Organism	QC Range (µg/mL)	Ref	Percent (%) in Range					
			Manual		WalkAway		autoSCAN-4	
			Turbidity	Prompt	Turbidity	Prompt	Turbidity	Prompt
E. coli ATCC 25922	0.03-0.12	100%	121/121 100%	121/121 100%	120/120 100%	121/121 100%	104/121 86.0%	103/121 85.1%
P. aeruginosa ATCC 27853	2-16	100%	121/121 100%	121/121 100%	121/121 100%	120/120 100%	121/121 100%	121/121 100%

•All results for ATCC 25922 *E. coli*, including those indicated as out of range for the AS4 (MIC ≤ 0.016 µg/mL), are 100% in range compared to the recently approved CLSI range for M100-ED30

CONCLUSION

This multicenter study showed that eravacycline MIC results for *Escherichia coli* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using EUCAST interpretive criteria.

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