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Updated CLSI Meropenem Breakpoints for MicroScan Dried Gram Negative MIC Panels from a Multicenter Assessment of *Enterobacterales* and *Pseudomonas aeruginosa*

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ABSTRACT

Objectives: Updated US FDA/CLSI meropenem breakpoints were evaluated against data from a multicenter clinical study with *Enterobacterales* and *P. aeruginosa* on a MicroScan Dried Gram Negative MIC (MSDGN) Panel. MIC results were compared to results obtained with frozen broth microdilution panels prepared according to CLSI methodology.
Materials/Methods: MSDGN panels were evaluated at four clinical sites by comparing MIC values obtained using the MSDGN panels to MICs utilizing a CLSI broth microdilution reference panel. The study included 738 *Enterobacterales* and *P. aeruginosa* clinical isolates tested using the turbidity and Prompt® methods of inoculation during the combined phases of efficacy and challenge. A subset of 11 organisms were 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 manually. Read times for the MSDGN panels were at 16-20 hours. Frozen reference panels were prepared and read according to CLSI methodology. FDA and CLSI breakpoints (µg/mL) used for interpretation of MIC results were: *Enterobacterales* ≤ 1 S, 2 I, ≥ 4 R; *P. aeruginosa* ≤ 2 S, 4 I, ≥ 8 R.
Results: Essential and categorical agreement were calculated compared to frozen reference panel results. Results for isolates tested during efficacy and challenge with Prompt inoculation and manual read are as follows:

Reporting Group	Essential Agreement (EA) %	Categorical Agreement (CA) %	Very Major Error (VMJ) %	Major Error (MAJ) %
Enterobacterales	95.5 (601/629)	98.4 (619/629)	0.0 (0/69)	0.0 (0/554)
P. aeruginosa	91.7 (99/108)	92.6 (100/108)	0.0 (0/25)	1.3 (1/77)

Reproducibility among the three sites was greater than 95% for all read methods for both the turbidity and Prompt inoculation methods.
Conclusion: Meropenem MIC results for *Enterobacterales* and *P. aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using updated FDA/CLSI interpretive criteria in this multicenter study.

INTRODUCTION

Data from a multicenter study evaluated the performance of a MicroScan Dried Gram Negative MIC panel with meropenem using *Enterobacterales* and *P. aeruginosa* isolates with FDA/CLSI interpretive breakpoints.

METHODS

Study Design: MicroScan Dried Gram Negative MIC 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 737 *Enterobacterales* and *P. aeruginosa* clinical isolates were tested among the four sites.
Quality Control Expected Results, https://www.fda.gov/STIC
Escherichia coli ATCC 25922: 0.008 – 0.06 µg/ml
Pseudomonas aeruginosa ATCC 27853: 0.12 - 1 µg/ml

METHODS (Continued)

Panels
Frozen reference and MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of meropenem 0.004 - 32 µg/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 25922 *E. coli* and ATCC 27853 *P. aeruginosa* (see https://www.fda.gov/STIC).

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±1ºC in the WalkAway system for 18 hours. Frozen reference panels were incubated in an off-line incubator. All dried panels were read by the WalkAway, autoSCAN-4, and manually.

Reproducibility
Reproducibility organisms with known results on-scale for meropenem 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 FDA/CLSI breakpoints for *Enterobacterales* and *P. aeruginosa*. (Table 1).

Table 1. Meropenem FDA/CLSI Interpretive Breakpoints (µg/ml) (https://www.fda.gov/STIC)

Organism Group	Susceptible	Intermediate	Resistant
Enterobacterales	≤ 1	2	≥ 4
P. aeruginosa	≤ 2	4	≥ 8

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 MIC 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 = 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.

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

RESULTS

Efficacy & Challenge Combined (Tables 2 and 3)
A total of 737 *Enterobacterales* and *P. aeruginosa* clinical isolates were tested among four sites.
Table 2. Efficacy & Challenge - Prompt
Essential Agreement for *Enterobacterales* and *P. aeruginosa* between MSDGN panel and frozen reference panel was 95.0% (700/737) for WalkAway System method, 94.8% (699/737) for autoSCAN-4 instrument, 95.0% (700/737) for manual read method using the Prompt inoculation method.
Categorical Agreement for *Enterobacterales* and *P. aeruginosa* between MSDGN panel and frozen reference panel was 97.7% (720/737) for WalkAway System method, 97.6% (719/737) for autoSCAN-4 instrument, 97.6% (719/737) 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	700/737	95.0	720/737	97.7	15/737	2.0	2/631	0.3	0/94	0.0
autoSCAN-4	699/737	94.8	719/737	97.6	16/737	2.2	1/631	0.2	1/94	1.1
Manual	700/737	95.0	719/737	97.6	17/737	2.3	1/631	0.2	0/94	0.0

During FDA review, additional isolates were assessed. The final FDA performance claims for WA read method with Prompt inoculation are as follows:

Read Method	No. Tested	Essential Agreement (%)	Categorical Agreement (%)
Enterobacterales	650	95.5	98.8
P. aeruginosa	131	93.1	92.4

Table 3. Efficacy & Challenge - Turbidity
Essential Agreement for *Enterobacterales* and *P. aeruginosa* between MSDGN panel and frozen reference panel was 96.5% (712/738) for WalkAway System method, 96.2% (710/738) for autoSCAN-4 instrument, 96.9% (715/738) for manual read method using the turbidity inoculation method.
Categorical Agreement for *Enterobacterales* and *P. aeruginosa* between MSDGN panel and frozen reference panel was 97.8% (722/738) for WalkAway System method, 97.8% (722/738) for autoSCAN-4 instrument, 97.6% (720/738) 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	712/738	96.5	722/738	97.8	13/738	1.8	2/631	0.3	1/95	1.1
autoSCAN-4	710/738	96.2	722/738	97.8	13/738	1.8	2/631	0.3	1/95	1.1
Manual	715/738	96.9	720/738	97.6	13/738	1.8	2/631	0.3	3/95	3.2

Reproducibility (Table 4)
A total of 11 isolates were tested for reproducibility at all three sites in triplicate over three days. During FDA review, 10 isolates were assessed resulting in ≥ 95% overall agreement for all inoculation and read methods.
Overall agreement (within ± one two-fold dilution) between all sites for the reproducibility phase was ≥ 95% for all combinations.

Table 4. Reproducibility Testing with MER – All Sites Combined

Read Method	Inoculation Method	No. (%) Agreement All Sites Combined
WalkAway	Prompt	289/297 (97.3)
autoSCAN-4		290/297 (97.6)
Manual		296/297 (99.7)
WalkAway	Turbidity	293/297 (98.7)
autoSCAN-4		295/297 (99.3)
Manual		297/297 (100)

Quality Control (Table 5)
QC results for the frozen reference panel were 98.4% in range for ATCC 25922 *E. coli* and 99.5% in range for ATCC 27853 *P. aeruginosa*.

Table 5. Quality Control

Organism	QC Range (mg/L)	WalkAway		autoSCAN-4		Manual	
		Prompt	Turbidity	Prompt	Turbidity	Prompt	Turbidity
E. coli ATCC 25922	0.008-0.06	97.5%	98.9%	97.5%	98.9%	98.5%	98.9%
P. aeruginosa ATCC 27853	0.12-1	100%	99.5%	100%	99.5%	100%	99.5%

The ability of the MicroScan Dried Gram Negative Panels to detect resistance to meropenem is unknown for the following species because an insufficient number of resistant strains were available at the time of comparative testing: *C. koseri and P. vulgaris.* Isolates yielding MIC results suggestive of a resistant interpretive category should be submitted to a reference laboratory.

Due to the occurrence of very major errors with meropenem and turbidity inoculation with all read methods, isolates of *K. pneumoniae* that provide an MIC of 1 µg/mL should be retested using an alternative/reference method.

CONCLUSION

Meropenem MIC results for *Enterobacterales* and *P. aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using updated FDA/CLSI interpretive criteria in this multicenter study. FDA cleared 14/NOV/2019.

†Deceased