Development of Aztreonam/Avibactam MIC Antimicrobial Susceptibility Test for Gram-negative Bacteria on MicroScan Dried Gram-negative MIC Panels

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ABSTRACT

Background: Development of an Aztreonam/Avibactam (AZA) antimicrobial susceptibility test was completed for the MicroScan Dried Gram-negative MIC (MSDGN) Panel. Test MIC results were compared to results obtained with frozen broth microdilution reference panels prepared according to CLSI methodology.

Materials/Methods: Development was conducted by comparing test MICs obtained using the MSDGN panel to MICs using a CLSI broth microdilution reference panel. A total of 1269 Enterobacterales and 120 *S. maltophilia* data points were tested at 16, 18, and 20 hour incubation times using the turbidity and Prompt® methods of inoculation. MSDGN panels were incubated at 35 ± 1°C and read on the WalkAway® System, the autoSCAN®-4 instrument, and visually. Frozen reference panels, prepared according to ISO/CLSI methodology, were inoculated using the turbidity inoculation method. All frozen reference panels were incubated at 35 ± 1ºC and read visually. Dilution sequence evaluated was 0.06/4 – 128/4 µg/mL.

Results: When compared to frozen reference panel MIC results, essential agreement for all isolates tested during development were as follows:

Read Method	Organism	Essential Agreement %	
		Turbidity	PROMPT
Manual Read	Enterobacterales	(1216/1269) 95.8%	(1217/1266) 96.1%
	S. maltophilia	(116/120) 96.7%	(96/99) 97.0%
WalkAway	Enterobacterales	(812/846) 96.0%	(760/844) 90.1%
	S. maltophilia	(78/80) 97.5%	(65/66) 98.5%
autoSCAN-4	Enterobacterales	(1217/1269) 95.9%	(1156/1266) 91.3%
	S. maltophilia	(119/120) 99.2%	(98/99) 99.0%

Conclusion: The development data showed that AZA MIC results for Enterobacterales and *S. maltophilia* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels. Essential agreement is ≥90% for all inoculation and read methods.

INTRODUCTION

MicroScan Dried Gram-Negative MIC panels were developed for testing of Gram-Negative bacteria with Aztreonam/Avibactam. Aztreonam/Avibactam is a novel investigational antibacterial agent created by combining the β-lactamase inhibitor avibactam and antibiotic aztreonam. It is suggested for the treatment of severe infections caused by metallo-beta-lactamase (MBL)-producing Enterobacterales[1]. Aztreonam and avibactam in combination maintain a broad range of activity while providing coverage against MBL producing isolates[1,2]. Data from a development study at Beckman Coulter evaluated the performance of a MicroScan Dried Gram-Negative MIC panel with Aztreonam/Avibactam using Enterobacterales and *S. maltophilia* isolates.

METHODS

Development Study Design:
MSDGN panels were tested with both turbidity and Prompt inoculation methods concurrently with CLSI frozen broth microdilution reference[3] panels. Isolates were set up in triplicate, incubated, and read at 16-, 18-, and 20-hours. WalkAway results do not include 20-hour incubation.

Panel builds
Frozen reference and MicroScan Dried Gram-Negative MIC panels contained two-fold doubling dilutions of AZA of 0.06 – 128 µg/mL for Aztreonam, with avibactam fixed at 4 µg/mL for each dilution in cation-adjusted Mueller-Hinton broth. Reference panels were prepared and frozen following CLSI M07 recommendations[3].

Quality Control and Ranges (µg/mL) per CLSI M100-ED33[4]

Organism	Organism Name	Turbidity
ATCC 25922	*E. coli*	0.03/4 - 0.12/4
ATCC 35218	*E. coli*	0.016/4 - 0.06/4
ATCC 700603*	*K. pneumoniae*	0.06/4 - 0.5/4
ATCC 27853	*P. aeruginosa*	2/4 - 8/4
Internally Developed QC		
ATCC 25668**	*P. aeruginosa*	2/4 - 8/4

*QC strain is recommended for routine QC
**ATCC 25668 is under development for purposes of providing on-scale MICs

Panel Inoculation, Incubation, and Reading
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, frozen reference panels were incubated in a non-CO_2 incubator for 16-20 hours or 20-24 hours when inoculated with Enterobacterales or *S. maltophilia* respectively. MSDGN MIC panels were incubated at 35±1ºC in the WalkAway system for 16- and 18- hours, and a non-CO_2 incubator for 20 hours. All dried panels were read manually and by the autoSCAN-4. Only the 16- and 18- hour panels were read by the WalkAway. A total of 1269/1266 Enterobacterales, and 120/99 *S. maltophilia* data points were collected from testing 423/422 Enterobacterales isolates, and 40/33 *S. maltophilia* isolates, with turbidity/prompt inoculation methods, respectively.

Data Analysis
Essential Agreement (EA) = MSDGN panel MIC within +/- 1 dilution of the frozen reference result MIC. EA acceptance criteria is considered to be ≥90%. Categorical Agreement (CA) was not evaluated because there are no CLSI, FDA or EUCAST breakpoints. Bias = For all isolates tested, a bias ≤30% is considered indicative of random variation. The following bias calculation is applied to the data per organism group: |(% test results above reference)–(% test results below references)|

RESULTS

Quality Control (Table 1), QC results for the test dried panel met acceptance criteria except for ATCC 27853 due to MIC results at 16/4 µg/mL with Prompt inoculation using the WalkAway and autoScan-4 read methods. *P. aeruginosa* ATCC 25668 provided on-scale MICs that were 100% in agreement with the range across all read and inoculation methods.

Table 1. Quality Control
AZA Dried test panel results with Prompt and turbidity inoculation methods:

Organism	WalkAway		autoSCAN-4		Manual	
	Prompt	Turbidity	Prompt	Turbidity	Prompt	Turbidity
ATCC 25922	(37/37) 100%	(37/37) 100%	(41/41) 100%	(41/41) 100%	(41/41) 100%	(44/44) 100%
ATCC 35218	(35/35) 100%	(35/35) 100%	(39/39) 100%	(40/40) 100%	(39/39) 100%	(43/43) 100%
ATCC 700603	(37/37) 100%	(37/37) 100%	(41/41) 100%	(41/41) 100%	(41/41) 100%	(44/44) 100%
ATCC 27853	(89/97) 91.8%	(92/97) 94.8%	(93/101) 92.1%	(96/101) 95.0%	(96/101) 95.0%	(100/104) 96.2%
ATCC 25668	(73/73) 100%	(73/73) 100%	(77/77) 100%	(77/77) 100%	(77/77) 100%	(77/77) 100%

Efficacy (Table 2), results for Enterobacterales and *S. maltophilia* met acceptance criteria with Prompt and turbidity. Overall EA for each read and inoculation method met acceptance criteria ≥90% with the exception of WalkAway 16-hour Prompt reads, which are slightly below 90% due to discrepancies with *Proteus*, *Providencia*, *Morganella*, and *Serratia* spp. When compared to frozen reference panel MIC results, essential agreement within ± 1 doubling dilution for all isolates tested during development were as follows:

Table 2. Efficacy
Development performance with the Prompt and turbidity inoculation methods:

Organisms	Read Method	Essential Agreement %					
		16 hrs		18 hrs		20 hrs	
		Prompt	Turbidity	Prompt	Turbidity	Prompt	Turbidity
Enterobacterales	Manual	412/422 97.6%	407/423 96.2%	405/422 96.0%	407/423 96.2%	400/422 94.8%	402/423 95.0%
	WalkAway***	376/422 89.1%	403/423 95.3%	384/422 91.0 %	409/423 96.7%	N/A	N/A
	autoSCAN-4	382/422 90.5%	405/423 95.7%	388/422 91.9%	409/423 96.7%	386/422 91.5%	403/423 95.3%
S. maltophilia	Manual	30/33 90.9%	37/40 92.5%	33/33 100%	39/40 97.5%	33/33 100%	40/40 100%
	WalkAway	32/33 97.0%	38/40 95.0%	33/33 100%	40/40 100%	N/A	N/A
	autoSCAN-4	32/33 97.0%	39/40 97.5%	33/33 100%	40/40 100%	33/33 100%	40/40 100%

***WalkAway results for Prompt 16 hr read without *Proteus*, *Providencia*, *Morganella*, and *Serratia* spp. is 301/312 (96.5%)

Bias (Table 3), No bias (≤30%) was demonstrated with all methods and organism groups except *S. maltophilia.* When not in agreement, results tended to be one doubling dilution lower for *S. maltophilia* with manual reads and both turbidity and Prompt inoculation methods.

Table 3. Bias
AZA Bias with the Prompt and turbidity inoculation methods

Organisms	Read Method	Bias %	
		Prompt	Turbidity
Enterobacterales	Manual	25.2	28.7
	WalkAway	8.6	16.5
	autoSCAN-4	9.0	20.8
S. maltophilia	Manual	46.5	40.8
	WalkAway	22.7	6.3
	autoSCAN-4	16.2	9.2

CONCLUSION

The development data showed that AZA MIC results for Enterobacterales and *S. maltophilia* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels. These development data supports the continued evaluation of MSDGN panel with AZA in a multicenter trial. The test MIC results of the reads done at 16-, 18-, and 20-hour incubation times were comparable to the frozen reference.

References

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