Reproducibility of Reference Broth Microdilution MICs When Testing Commonly Encountered Bacterial Pathogens

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Introduction

Minimum inhibitory concentration (MIC) testing by broth microdilution (BMD) per Clinical and Laboratory Standards Institute M07-A10 is the gold standard method for commercial antimicrobial susceptibility test system evaluation for clearance by the U.S. Food and Drug Administration. The precision of BMD MICs measured during the Accelerate PhenoTest™ BC kit FDA clinical trial was evaluated.

Methods

- A total of 3,317 isolates of *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus* spp. and *Enterococcus* spp. were received from 12 clinical sites.
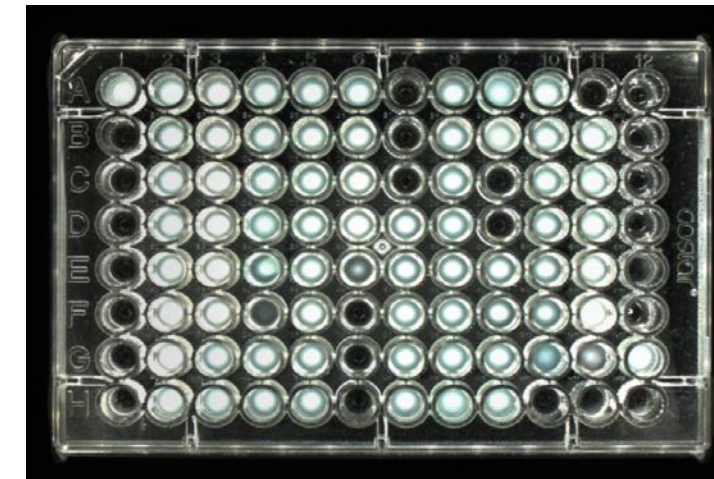
- BMD (**Figure 1**) was performed in triplicate according to CLSI M07-A10 using frozen panels prepared in-house.

Figure 1. BMD plate.

- For BMD inocula preparation, 3 separate McFarland 0.5 suspensions were prepared for each isolate from colonies on an overnight blood agar plate (BAP). These were combined into a single suspension, which was subsequently diluted and used to inoculate 3 MIC panels.

- Two highly trained technologists performed manual reads daily (n=7 technologists in total performed reads). Results from a digital BMD imaging system (Biotek) were also examined.

- Individual MIC results were compared to the modal MIC obtained from the 3 results for each drug/isolate combination, and absolute agreement (AA) and essential agreement (EA, i.e., MIC +/- 1 $\log_2$ dilution) were evaluated. If no mode was obtained, triplicate BMD was repeated and the mode taken from the 6 results. Results for intrinsic resistance were excluded from the data set.

Results

- From the 3,317 isolates, 99,829 individual MICs were assessed. For 4.1% of reads, a mode could not be established.

- By organism, EA ranged from 90.7-98.5% and AA ranged from 77.9-95.6% (**Table 1**).

- By antimicrobial, EA ranged from 91.6-99.3% and AA ranged from 79.6-97.7% (**Table 2**).

Results

Table 1. Reproducibility and agreement by organism.

Organism	-3	-2	-1	0	1	2	3	No MIC	Total	EA %	AA %
Gram-Positive											
S. aureus	7	4	455	17111	474	8	21	239	18319	98.5	93.4
S. lugdunensis	0	0	24	1143	21	0	2	35	1225	97.0	93.3
CoNS	3	10	226	11526	393	25	18	150	12351	98.3	93.3
E. faecalis	4	4	121	6530	194	7	9	84	6953	98.5	93.9
E. faecium	4	1	53	4778	93	6	3	62	5000	98.5	95.6
Gram-Negative											
E. coli	3	34	893	18694	974	101	15	438	21152	97.2	88.4
Klebsiella spp.	2	29	472	9627	498	47	8	402	11085	95.6	86.9
Enterobacter spp.	2	14	215	3877	270	30	10	340	4758	91.7	81.5
Citrobacter spp.	0	5	88	1966	137	13	0	90	2299	95.3	85.5
Proteus spp.	6	7	107	2278	123	21	18	56	2616	95.9	87.1
S. marcescens	2	10	159	1992	168	23	6	196	2556	90.7	77.9
P. aeruginosa	0	8	143	3472	161	11	4	87	3886	97.2	89.4
A. baumannii	6	8	188	6981	273	31	5	137	7629	97.6	91.5
Total	39	134	3144	89975	3779	323	119	2316	99829	97.1	90.1

Table 2. Reproducibility and agreement by antibiotic.

Antibiotic	-3	-2	-1	0	1	2	3	No MIC	Total	EA %	AA %
Gram-Positive											
Ampicillin	3	5	136	5027	227	27	6	86	5517	97.7	91.1
Ceftaroline	1	4	82	5381	115	3	5	85	5676	98.3	94.8
Doxycycline	3	4	110	5001	154	2	2	72	5348	98.5	93.5
Erythromycin	1	0	55	5526	92	2	9	89	5774	98.3	95.7
TMP-SMX	8	2	34	5194	51	4	26	0	5319	99.3	97.7
Daptomycin	1	3	195	5081	208	7	1	87	5583	98.2	91.0
Linezolid	1	1	112	4404	228	0	4	66	4816	98.5	91.5
Vancomycin	0	0	155	5474	100	1	0	85	5815	98.5	94.1
Gram-Negative											
Amp-Sul	0	1	131	2541	142	6	2	48	2871	98.0	88.5
Pip-Tazo	1	8	170	3139	231	32	15	128	3724	95.1	84.3
Cefazolin	0	3	111	1769	93	11	0	50	2037	96.9	86.8
Cefepime	5	2	61	3685	64	9	3	99	3928	97.0	93.8
Ceftazidime	0	14	173	3079	287	37	6	163	3759	94.2	81.9
Ceftriaxone	1	9	134	2271	197	21	3	141	2777	93.7	81.8
Ertapenem	2	1	54	2809	53	8	7	87	3021	96.5	93.0
Imipenem	3	2	60	3744	86	4	1	62	3962	98.2	94.5
Meropenem	1	2	86	3640	92	8	6	75	3910	97.7	93.1
Amikacin	1	17	288	3169	303	45	1	157	3981	94.5	79.6
Gentamicin	0	10	239	3366	242	18	1	126	4002	96.1	84.1
Tobramycin	0	9	250	3350	243	15	5	76	3948	97.3	84.9
Ciprofloxacin	1	3	42	3865	30	2	1	98	4042	97.4	95.6
Minocycline	3	4	146	2654	215	18	2	69	3111	96.9	85.3
Aztreonam	2	13	116	2939	106	9	3	127	3315	95.4	88.7
Colistin	1	17	204	2867	220	34	10	240	3593	91.6	79.8
Total	39	134	3144	89975	3779	323	119	2316	99829	97.1	90.1

Results

Table 3. Reproducibility and agreement by organism, intermediate modes.

Organism	-3	-2	-1	0	1	2	3	No MIC	Total	EA %	AA %
Gram-Positive											
S. aureus	0	0	1	100	2	0	0	-	103	100	97.1
S. lugdunensis	-	-	-	-	-	-	-	-	-	-	-
CoNS	0	0	1	55	2	1	1	0	60	96.7	91.7
E. faecalis	0	0	1	241	11	0	0	4	257	98.4	93.8
E. faecium	0	0	0	53	9	1	0	0	63	98.4	84.1
Gram-Negative											
E. coli	0	2	52	503	21	4	3	16	601	95.8	83.7
Klebsiella spp.	0	1	22	231	25	1	0	13	293	94.9	78.8
Enterobacter spp.	0	1	10	103	11	1	0	7	133	93.2	77.4
Citrobacter spp.	0	0	3	34	3	0	0	0	40	100	85
Proteus spp.	0	0	2	48	1	1	1	0	53	96.2	90.6
S. marcescens	0	0	3	26	6	2	1	1	39	89.7	66.7
P. aeruginosa	0	0	6	195	19	2	0	0	222	99.1	87.8
A. baumannii	1	0	13	247	18	4	0	4	287	96.9	86.1
Total	1	4	114	1836	128	17	6	45	2151	96.6	85.4

Table 4. Reproducibility and agreement by technologist.

	-3	-2	-1	0	1	2	3	No MIC	Total	EA %	AA %
Reader 1	2	6	74	3308	147	2	1	101	3641	96.9	90.9
Reader 2	2	2	87	8421	340	3	6	223	9084	97.4	92.7
Reader 3	91	70	928	21068	615	18	15	1071	23876	94.7	88.2
Reader 4	10	15	184	4128	160	6	5	287	4795	93.3	86.1
Reader 5	44	65	1079	19548	746	19	8	1014	22523	94.9	86.8
Reader 6	54	105	1409	26288	817	30	31	1274	30008	95.0	87.6
Reader 7	7	9	79	5362	303	10	2	130	5902	97.3	90.9
Total	210	272	3840	88123	3128	88	68	4100	99829	95.3	88.3

- When only isolates with modal MICs in the intermediate interpretive category were evaluated (n=590), EA was 89.7-100% and AA was 66.7-97.1% (**Table 3**).

- Precision of technologists ranged from 93.3-97.4% EA and 86.1-92.7 AA. EA of instrument readings was 95.3% and AA was 88.3% (**Table 4**).

Conclusion

Under the controlled environment of a standardized reference laboratory using highly trained technologists, precision with both manual and instrument MIC readings from BMD panels was within the expected >90% EA. However, EA and AA was poorer for isolates with MICs in the intermediate category.