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Reproducibility of the Accelerate ID/AST Blood Culture Assay at Multiple Clinical Sites

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AMENDED ABSTRACT

Background: Interlaboratory reproducibility of antimicrobial susceptibility results has been identified by CLSI and CAP as a significant challenge. In this multi-center study, we evaluated the reproducibility of a novel integrated ID and AST system that can provide molecular-based ID and automated microscopy-based phenotypic AST results directly from positive blood cultures.

Methods: Five bacterial isolates (*S. aureus*, *E. faecalis*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*) were tested at 9 clinical sites. Isolates were seeded into BacT/ALERT® SA Standard Aerobic, BACTEC™ Plus Aerobic/F Medium or VersaTREK® Redox 1 Aerobic Media with healthy donor blood, incubated until positivity, and run on the Accelerate Pheno™ system. ID agreement and AST reproducibility for 24 antibiotics (amikacin, ampicillin, ampicillin-sulbactam, aztreonam, cefazolin, cefepime, ceftaroline, ceftazidime, ceftriaxone, ciprofloxacin, colistin, daptomycin, doxycycline, ertapenem, erythromycin, gentamicin, imipenem, linezolid, meropenem, minocycline, piperacillin-tazobactam, tobramycin, trimethoprim-sulfamethoxazole and vancomycin) and 4 resistance phenotypes (MRSA/MRS, MLSb, high-level gentamicin and streptomycin) were determined.

Results: Overall ID sensitivity and specificity were 97.8% (44/45) and 100% (765/765), respectively, with 1 false-negative *S. aureus* result. Interlaboratory AST reproducibility (MIC values within 1 dilution of the mode) was 98.4% (443/450).

Conclusions: The Accelerate ID/AST Blood Culture Assay produced highly reproducible ID and AST results between all clinical laboratory sites using several types of blood culture systems.

INTRODUCTION

The reproducibility of a novel rapid ID and AST system was tested across multiple clinical sites.

METHODS

For each isolate, 10 to 100 CFU were spiked into blood culture bottles (BacT/ALERT® SA Standard Aerobic, BACTEC™ Plus Aerobic/F Medium or VersaTREK® Redox 1 Aerobic Media) along with 8-10 mL healthy donor blood, and incubated in their respective blood culture systems until they signaled positive. Positive blood culture samples were loaded and run on the fully-automated Accelerate Pheno™ system using the Accelerate PhenoTest™ BC kit according to manufacturer's instructions (Figure 1). Sample cleanup was performed using automated gel electrofiltration prior to automatically dispensing sample aliquots into separate flowcells of a disposable test cassette. Electrokinetic concentration used a low voltage applied for 5 min to capture bacterial cells on the transparent lower surface of each inoculated flowcell channel prior to identification by fluorescence *in situ* hybridization (FISH).

Following FISH ID and a 1 h pre-growth step, antimicrobial susceptibility testing (AST) was performed using a single concentration of each test antibiotic in cation-adjusted Mueller-Hinton broth with 0.85% agar in separate flowcells. Automated microscopy captured time-lapse images every 10 min for up to 4.5 h and analyzed growth features of each immobilized progenitor cell as it grew into a clone of daughter cells in the presence or absence of antibiotic (Figure 2). Proprietary software algorithms converted these growth features into a minimum inhibitory concentration (MIC) value or positive or negative resistance phenotype test result.

For ID results, sensitivity and specificity were calculated compared to the expected ID result.

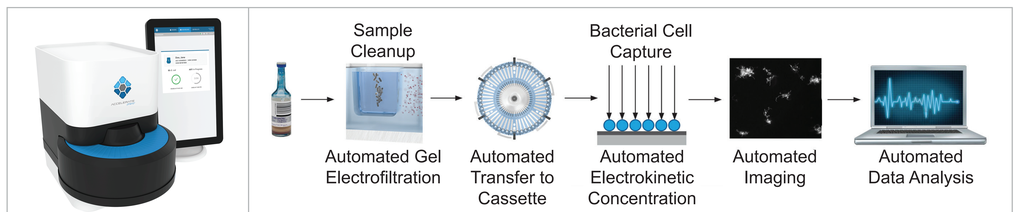

Figure 1: *Accelerate Pheno™ system (left). Study process flow (right) including simulated blood culture preparation, sample cleanup by automated gel electrofiltration, automated transfer to the cassette for bacterial immobilization by electrokinetic concentration, automated imaging, and automated data analysis.*

For AST results, the test mode was calculated as the most frequent test result. The differences between the test results and test mode in MIC doubling dilutions were calculated for each antibiotic-organism combination.

For resistance phenotype tests, positive or negative results that matched the test mode were assigned a difference of 0.

Between-site reproducibility was calculated as the total number of results that fell within one doubling dilution of the test mode divided by the total number of results.

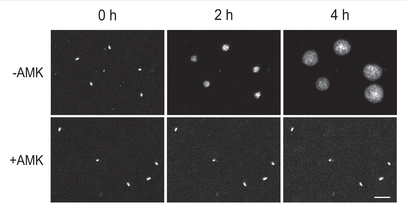

Figure 2: *Time-lapse images of amikacin-susceptible K. pneumoniae growing in the presence (+AMK) and absence (-AMK) of amikacin at 0, 2 and 4 hours. Images zoomed in to view individual bacterial clones. Scale bar in the lower right image is 20 µm.*

RESULTS

For identification results, overall sensitivity and specificity were calculated across all 18 target probes. ID results by target probe are shown in Table 1. All of the tested probes had 100% sensitivity and specificity except for the *Staphylococcus aureus* probe, which had 1 false-negative result.

Table 1: *Sensitivity and specificity by target ID probe.*

Organism (Isolate)	Sensitivity*	Specificity**
Enterococcus faecalis	9/9 (100%)	36/36 (100%)
Staphylococcus aureus	8/9 (88.9%)	36/36 (100%)
Acinetobacter baumannii	9/9 (100%)	36/36 (100%)
Klebsiella spp.	9/9 (100%)	36/36 (100%)
Pseudomonas aeruginosa	9/9 (100%)	36/36 (100%)

*Sensitivity for each of the remaining 13 target ID probes was inevaluable (0/0).
**Specificity for each of the remaining 13 target ID probes was 45/45 (100%).

For antimicrobial susceptibility testing results, overall between-site reproducibility was 98.4%. Because of the challenge of selecting a single isolate with on-scale results for all 24 antibiotics and 4 resistance phenotypes tested, off-scale results were indicated, but reproducibility was calculated with off-scale results taken as the low or high reportable range value only. Reproducibility results by individual antibiotic-organism combinations are shown in Table 2.

CONCLUSION

The Accelerate Pheno™ system and Accelerate PhenoTest™ BC kit showed very high ID and AST reproducibility directly from positive blood cultures for the 5 isolates tested across the 9 clinical trial sites.

For investigational use only. The performance characteristics of this product have not been established.

Table 2: *Reproducibility results by antibiotic and organism, pooled across sites. Results are shown as the difference in MIC doubling dilutions between the test result and the test mode. The study is based on 9 organisms tested at 9 sites across 24 antibiotics and 4 resistance phenotypes. The low and high reportable ranges for each antibiotic-organism combination are shown. Off-scale results are shown in blue font.*

	Difference in MIC doubling dilutions between test result and test mode											
Antibiotic/Organism	**-4**	**-3**	**-2**	**-1**	**0**	**+1**	**+2**	**+3**	**+4**	**Test Mode**	**Low Range**	**High Range**
Amikacin												
Acinetobacter baumannii				1	4	4				64	4	128
Klebsiella pneumoniae					9					4	4	128
Pseudomonas aeruginosa				2	4	2		1		16	4	128
Ampicillin												
Enterococcus faecalis					9					2	2	32
Ampicillin-Sulbactam												
Acinetobacter baumannii					9					64	2	64
Klebsiella pneumoniae				1	8					32	2	64
Aztreonam												
Klebsiella pneumoniae					9					2	1	32
Pseudomonas aeruginosa				3	6					32	2	64
Cefazolin												
Klebsiella pneumoniae					9					8	0.5	16
Cefepime												
Acinetobacter baumannii					9					64	2	64
Klebsiella pneumoniae					9					1	1	32
Pseudomonas aeruginosa					7	2				16	2	64
Cefoxitin												
Staphylococcus aureus					9					POS		
Ceftaroline												
Staphylococcus aureus				2	7					1	0.25	8
Ceftazidime												
Klebsiella pneumoniae					8	1				2	1	32
Pseudomonas aeruginosa			1		8					16	2	64
Ceftriaxone												
Klebsiella pneumoniae					9					0.5	0.25	8

	Difference in MIC doubling dilutions between test result and test mode											
Antibiotic/Organism	**-4**	**-3**	**-2**	**-1**	**0**	**+1**	**+2**	**+3**	**+4**	**Test Mode**	**Low Range**	**High Range**
Ciprofloxacin												
Acinetobacter baumannii					9					8	0.25	8
Klebsiella pneumoniae				1		8				1	0.25	8
Pseudomonas aeruginosa				1	2	6				1	0.25	8
Colistin												
Acinetobacter baumannii						7	2			0.5	0.5	8
Klebsiella pneumoniae						9				0.5	0.5	8
Pseudomonas aeruginosa			1	2	6					4	0.5	16
Daptomycin												
Enterococcus faecalis					9					1	1	8
Staphylococcus aureus					5	4				0.25	0.25	2
Doxycycline												
Enterococcus faecalis				1	7	1				8	1	32
Staphylococcus aureus				1	6	2				16	1	32
Ertapenem												
Klebsiella pneumoniae				2	4	3				1	0.125	4
Erythromycin												
Staphylococcus aureus					9					16	0.125	16
Gentamicin												
Klebsiella pneumoniae					9					1	1	32
Pseudomonas aeruginosa					8	1				8	1	32
High-Level Gentamicin												
Enterococcus faecalis					9					NEG		
High-Level Streptomycin												
Enterococcus faecalis					9					POS		
Imipenem												
Acinetobacter baumannii				2	7					16	0.5	16

	Difference in MIC doubling dilutions between test result and test mode											
Antibiotic/Organism	**-4**	**-3**	**-2**	**-1**	**0**	**+1**	**+2**	**+3**	**+4**	**Test Mode**	**Low Range**	**High Range**
Pseudomonas aeruginosa					9					4	0.5	16
Linezolid												
Enterococcus faecalis					9					2	0.5	16
Staphylococcus aureus					9					2	1	16
Meropenem												
Acinetobacter baumannii					9					16	0.5	16
Klebsiella pneumoniae					5	3		1		0.25	0.25	8
Pseudomonas aeruginosa				2	6	1				8	0.5	16
Minocycline												
Acinetobacter baumannii					6	3				8	1	32
MLSB												
Staphylococcus aureus					9					POS		
Piperacillin-Tazobactam												
Acinetobacter baumannii					9					256	4	256
Klebsiella pneumoniae				4	4				1	8	4	256
Pseudomonas aeruginosa					6	3				64	4	256
Trimethoprim-Sulfamethoxazole												
Staphylococcus aureus					9					1	0.5	8
Tobramycin												
Klebsiella pneumoniae					8	1				1	1	32
Pseudomonas aeruginosa				1	8					2	1	32
Vancomycin												
Enterococcus faecalis					8	1				1	1	64
Staphylococcus aureus					8	1				0.5	0.5	32
Total	**0**	**0**	**4**	**26**	**382**	**35**	**0**	**2**	**1**			
Between-Site Reproducibility	**443/450 (98.4%)**											