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Performance of the Accelerate Diagnostics Pheno™ system with resin-containing BacT/ALERT® Plus blood culture bottles

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ABSTRACT

Bacteremia and septicemia require rapid identification (ID) and antimicrobial susceptibility testing (AST) to start targeted, appropriate therapy. To answer this need, Accelerate Diagnostics, Inc., developed the Accelerate Pheno™ system (AXDX), a fast ID and phenotypic AST platform. Performance of a pre-FDA clearance version of AXDX was evaluated using 261 positive BacT/ALERT® Plus bottles and compared with standard of care (SOC). Average time to ID was reduced by 24.9±6.9 h and AST by 36.7±18.9 h compared with SOC. AXDX reports ID and AST of blood pathogens in 1.9 and 7.1 h. Positive percent agreement and negative percent agreement of AXDX ID were 94.5% and 98.9%, respectively. AXDX AST had an essential agreement of 96.5% and categorical agreement of 94.6% with 4 major errors and 7 very major errors. AXDX performance was acceptable for all 3 bottle types. Rapid ID and AST with AXDX could impact patient care and antimicrobial stewardship.

1. Introduction

Septicemia was the 10th leading cause of death for women in the United States and the 11th leading cause overall for the United States population in 2014 (Kochanek et al., 2016). Septicemia also accounted for the second leading cause of hospital deaths in 2010 (based on first listed ICD-9 coded diagnosis) (Hall et al., 2013) and the most expensive condition (at approximately $23,000,000,000 for all payers as an aggregate) treated in U.S. hospitals in 2013 (Torio and Moore, 2016). Furthermore, the impact of sepsis on mortality and morbidity has been shown to extend at least 2 years beyond the time a patient is hospitalized (Winters et al., 2010). These observations illustrate the importance of developing strategies to lessen the mortality and financial impact of sepsis (Rhodes et al., 2017).

Following the development of septic hypotension, the time to initiation of appropriate antimicrobial therapy significantly affects in-hospital mortality (Kumar et al., 2006). If initiated within the first hour, the survival rate is 79.9% but decreases by an average of 7.6%/h over the next 6 h. Initiation of inappropriate empiric therapy can result in a 5-fold decrease in survival compared to initiation of appropriate therapy (Kumar et al., 2009).

The Surviving Sepsis Campaign guidelines highlight the critical nature of appropriate therapy and addressing the source of infection within the first 12 h of symptom onset (Rhodes et al., 2017). Key to achieving this goal is recognition of the etiologic agent and its antimicrobial susceptibility profile. Such recognition is also applicable to patients with bacteremia or fungemia because, similar to sepsis, outcomes are affected by the length of delay before starting appropriate therapy (Fraser et al., 2006; Ibrahim et al., 2000; Kang et al., 2005; Perez et al., 2014; Zaragoza et al., 2003).

Methods of diagnosing bloodstream infections and performing susceptibility testing rely on obtaining a positive blood culture after an extended incubation time, a laborious process that can take multiple days (~2–5 days) (Kirn et al., 2014; Lamy et al., 2016) and does not provide a physician with timely antimicrobial susceptibility information to implement targeted therapy. Without rapid identification (ID) and susceptibility data, clinicians often rely on empiric broad-spectrum antibiotic therapy until susceptibility is determined. Initiation of inappropriate, broad-spectrum antimicrobial use can drive antibiotic and negatively impact patient outcomes (Fair and Tor, 2014; Ventola, 2015).

Several new technologies [including BioFire FilmArray® with the BCID panel (bioMérieux Inc., Durham, NC) and the Luminex Verigene® with its BC-GP and Gram- BC-GN panels (Austin, TX)] have been developed to rapidly (<6 h) detect bacterial and fungal agents of bloodstream infection and identify a subset of antibacterial resistance genes directly from positive blood cultures. These molecular technologies have proven useful for influencing antimicrobial treatment, as long as these results are acted upon in a timely manner (Box et al., 2015; Felsenstein et al.,

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2016; MacVane and Nolte, 2016; Timbrook et al., 2015; Walker et al., 2016). Current molecular assays for blood culture ID and resistance gene detection target a limited set of resistance genes, necessitating subsequent phenotypic antimicrobial susceptibility testing (AST).

The Accelerate Pheno™ system (AXDX) is the first multiplex system to provide ID within 90 min and minimum inhibitory concentration (MIC)–based phenotypic susceptibility data within approximately 7 h of bottle positivity. AXDX uses automated sample prep consisting of gel electrofiltration and microbial cell capture on a charged surface, followed by ID using fluorescence *in situ* hybridization with universal and targeted probes. Based on the identification, the MIC for a chosen set of antibiotics is determined through imaging the growth response to each antimicrobial, resulting in MICs that are interpreted as susceptible (S), intermediate (I), or resistant (R). AXDX can identify 28 unique microorganisms and contains a panel of 18 ASTs and 2 resistance phenotype tests. The benefits of short hands-on time and preparation work along with fast and multiplex capabilities make the Accelerate Pheno™ system an attractive technology to combat sepsis and improve patient care.

Several recent studies evaluating AXDX have been published, but none of these utilized the bioMérieux resin-containing blood culture bottles which became commercially available in the United States in 2013 (Charnot-Katsikas et al., 2018; Lutgring et al., 2018; Marschal et al., 2017; Pancholi et al., 2018; Pantel et al., 2018). Because the resin may interfere with the analysis performed by AXDX and because of the possibility that other products from the bottles could interfere with system performance, we prospectively evaluated a pre–FDA-cleared version of the Accelerate Pheno™ system using clinical positive blood cultures incubated in bioMérieux resin bottles. This was the first time performance was demonstrated with this bottle type.

2. Materials and methods

2.1. Study design

Blood cultures were collected using bioMérieux BacT/ALERT® Plus resin-containing media bottles (BacT/ALERT FA Plus, FN Plus, and PF Plus) between April 14, 2016, and February 11, 2017. All bottles were incubated in the BacT/ALERT 3D system (bioMérieux, Durham, NC). We included Gram-negative and Gram-positive organisms, as well as yeast. Following routine Gram stain, routine subculturing included ID and AST. ID was performed by MALDI-TOF MS (KnowledgeBase 2.0, VITEK® MS, bioMérieux) and supplemented with other ID methods including the MicroScan® ID/AST combination panels (Beckman Coulter, Carlsbad, CA) and biochemicals.

Each test sample was placed on the Accelerate Pheno™ system within 8 h of positivity on the BacT/ALERT® system. Samples were enrolled sequentially based on module availability. We did not test repeat blood cultures from the same patient drawn on the same day. Briefly, positive blood culture bottles were vortexed for 10 s, and 5 mL of blood was removed using a 10-mL syringe with a 21-gauge needle attached. Blood was added to the Accelerate PhenoTest™ BC kit sample vial and placed in the test cartridge. Total hands-on time was about 2 min. Reagents, cartridges, and cassettes were stored according to instructions provided by Accelerate Diagnostics, Inc. Five quality control bacterial samples were run every week. Two modules were alternated during the study. The AXDX software used was version 1.0, and reports were automatically generated. For statistical purposes, a time of 30 min was assumed between the bottle being flagged positive on the BacT/ALERT® system and sample insertion into Accelerate Pheno™ system (similar to standard practice of inoculating subcultures). Results from the Accelerate Pheno™ system were compared with the standard of care (SOC) practice. ID results were concordant if the same genus–species resulted from both methods.

AST SOC was performed using MicroScan® combination panels. In 2 cases, MIC was obtained using ETEST® (bioMérieux, Durham, NC). The Accelerate Pheno™ system MIC and interpretation (S, I, R) were compared with the SOC result. Essential agreement (EA) was reached in cases where the AXDX MIC was within 1 dilution of the SOC MIC. Categorical agreement (CA) was determined based on agreement of the S, I, and R interpretation. Discrepant results were categorized into minor errors (I vs. S or R), major errors (false resistance), and very major errors (false susceptibility). Clinical and Laboratory Standards Institute (CLSI) document M100S, 26th edition breakpoints were used (CLSI, 2016) for all antimicrobials except colistin which were based on European Committee on Antimicrobial Susceptibility Testing version 6.0 breakpoints.

This study was approved by the Duke Medicine Review Board for Clinical Investigations under study number Pro00062856.

2.2. Discrepancy testing

Discrepancy testing for ID was not performed as the original blood culture samples were not retained and therefore unavailable for repeat testing on the Accelerate Pheno™ system. Discrepancy testing for AST was performed by broth microdilution in accordance with CLSI document M07-A10 (CLSI, 2015) except for methicillin-resistant staphylococci, which used the CLSI disk diffusion cefoxitin screen (CLSI, 2016). Isolates subjected to discrepancy testing were kept frozen at −80 °C until testing (Table A.2).

2.3. Statistical analysis

PPA (positive percent agreement) and NPA (negative percent agreement) were calculated for Accelerate Pheno™ system ID results compared with SOC to assess ID accuracy. Only CA, very major error, and major error rates for Accelerate Pheno™ system versus Microscan®/ETEST® AST were calculated for resistance phenotype tests (MRSA by cefoxitin resistance, MLSb for coagulase-negative staphylococci).

Timing calculations compared the average times to ID and AST results between AXDX and SOC. Statistical significance was determined using a Welch 2-sample *t* test implemented in R version 3.4.1.

2.4. Exclusions

Positive blood culture samples that were more than 8 h old were excluded from analysis. Other samples excluded from the study were those with technical and ID failures. AST failures were excluded from AST analysis.

3. Results

A total of 261 blood cultures were tested by the Accelerate Pheno™ system and SOC methods. After exclusions, 237 samples remained for analysis (Table A.1). There were 6 technical failures, where the instrument itself or the software prevented reporting of ID and AST results. There were 13 ID failures, when too few cells are present in the control channel for ID analysis. No ID or AST results were reported in these cases, and the following was printed on the report: “No ID results reported: too few cells for analysis” (CLSI, 2016). Of the ID runs that proceeded to AST, there were 5 partial and 13 total AST failures. For the latter, this was frequently due to a growth control failure in which the organism in the growth control flow cell channel fell out of the expected growth pattern.

3.1. ID Performance

A total of 237 samples were evaluated for ID performance. A total of 241 organisms were detected, including 23 not detected by the panel. For the remaining 218 on-panel organisms, the ID performance was determined for 155 Gram-positive organisms, 57 Gram-negative organisms, and 6 yeasts. *Citrobacter* spp. were lacking in our study.

Table 1
Accelerate Pheno™ system PPA and NPA characteristics for organism identification.

Microbe	PPA[a]		NPA[b]	
Gram-positive				
Staphylococcus aureus	100%	65/65	93.3%	154/165
Staphylococcus lugdunensis	100%	5/5	100%	226/226
Coagulase-negative *Staphylococcus* spp.	93.1%	54/58	96%	166/173
Enterococcus faecalis	100%	8/8	100%	229/229
Enterococcus faecium	100%	7/7	99.1%	227/229
Streptococcus spp.	66.7%	8/12	96.4%	217/225
Gram-negative				
Escherichia coli	93.1%	27/29	99%	206/208
Klebsiella spp.	100%	10/10	98.7%	223/226
Enterobacter spp.	100%	2/2	100%	234/234
Proteus spp.	100%	3/3	99.6%	233/234
Citrobacter spp.	NA%	0/0	99.6%	236/237
Serratia marcescens	100%	6/6	100%	231/231
Pseudomonas aeruginosa	75%	3/4	100%	233/233
Acinetobacter baumannii	100%	3/3	100%	234/234
Yeast				
Candida albicans	0%	0/1	99.6%	234/235
Candida glabrata	100%	5/5	98.7%	229/232
Total				
Gram-positive	94.8%	147/155	97.8%	1219/1247
Gram-negative	94.7%	54/57	99.6%	1830/1837
Yeast	83.3%	5/6	99.1%	463/467
All	94.5%	206/218	98.9%	3512/3551

[a] Four Gram-positive samples with indeterminate results were excluded from analysis
[b] Nineteen samples with indeterminate results were excluded from analysis (16 Gram-positive, 2 Gram-negative, 1 yeast)

For ID of Gram-positive organisms, PPA and NPA were 100% for *E. faecalis* and *S. lugdunensis*. PPA and NPA >90% were seen for coagulase-negative *Staphylococcus* spp., *E. faecium*, and *S. aureus*. The only low-scoring Gram-positive organisms were *Streptococcus* spp., which showed only 66.7% PPA (Table 1).

Likewise, Gram-negative organisms demonstrated 100% PPA and NPA for identification of *A. baumannii, Enterobacter* spp., and *S. marcescens*. The Accelerate Pheno™ system achieved >90% PPA and NPA for all organisms except *P. aeruginosa*, which had 100% NPA but only 75% PPA. Overall, Accelerate Pheno™ system exhibited 94.8% PPA and 97.8% NPA for Gram-positives and 94.7% PPA and 99.6% NPA for Gram-negatives.

For the 6 yeast samples, PPA and NPA for the Accelerate Pheno™ system with *C. glabrata* were 100% and 98.7%, respectively. Due to an undetected *C. albicans* sample, the Accelerate Pheno™ system had lower PPA for yeasts (83.3%) compared to bacteria but exhibited 99.1% NPA. For all organisms, the Accelerate Pheno™ system demonstrated an overall PPA of 94.5% and NPA of 98.9% (Table 1).

Of the 237 runs analyzed for ID, 4 samples contained more than 1 species. Polymicrobial samples are shown in Table 2.

3.2. AST performance of the Accelerate Pheno™ system compared to SOC

AST results were generated by AXDX for 137 organisms (87 Gram-positive and 50 Gram-negative) and compared with SOC methods. All 6 very major errors reported by the Accelerate Pheno™ system were with erythromycin. These 6 false susceptibility results out of a total of 33 erythromycin-resistant tests resulted in an 18.2% very major error rate for erythromycin. When calculating the overall VME rate across all antibiotics, the rate was 8.1% (6/74). We observed an overall major error in 4 out of 696 susceptibility results (0.6%). Minor errors were observed in 33 out of 790 total susceptibility results, resulting in a rate of 4.2% (Table 3). Table A.2 shows discrepancy testing results.

To examine the resistance mechanisms reported by the Accelerate Pheno™ system, we compared the resistance of staphylococci. We had 42 cases of *Staphylococcus aureus* species that had a cefoxitin result by the MicroScan® WalkAway® system. Of these, we encountered 1 very major error and no major errors, resulting in a CA of 97.6%.

The Accelerate Pheno™ system produced AST results for 6 cases of *E. faecium* bacteremia. Of these, 2 tested resistant to both ampicillin and vancomycin by our SOC methods as well as the Accelerate Pheno™ system. There were no very major errors observed with these drug–organism combinations. *E. faecalis* was found in 8 samples, but expectedly, none of these expressed resistance to ampicillin or vancomycin. The Accelerate Pheno™ system called resistance profiles accurately for enterococci.

The Accelerate Pheno™ system correctly called the resistance pattern of the 7 of 25 blood cultures with ciprofloxacin-resistant *E. coli* and 5 of 26 cultures with ampicillin-sulbactam–resistant *E. coli*. All 7 *Klebsiella* spp. that were ceftriaxone-susceptible by SOC were called susceptible by AXDX.

For *S. marcescens*, the Accelerate Pheno™ system provided 100% CA for ciprofloxacin (6/6) and ceftazidime (4/4) and 80% CA for tobramycin (4/5). For *P. aeruginosa*, the Accelerate Pheno™ system correctly reported piperacillin-tazobactam susceptibility in 2 out of 3 cases. Table A.3 shows the antimicrobial susceptibility profiles for 3 isolates with multidrug resistance.

Table 2
Accelerate Pheno™ system versus SOC ID results for polymicrobial samples.

SOC ID result	AXDX ID result
Streptococcus mitis, Streptococcus oralis	N/A
Staphylococcus epidermis, Bacteroides fragilis	Coagulase-negative *Staphylococcus* spp.
Escherichia coli, Klebsiella pneumoniae	*Escherichia coli, Klebsiella* spp.
Escherichia coli, Klebsiella pneumoniae	*Klebsiella* spp.

Table 3
Antimicrobial susceptibility testing performance (postdiscrepancy testing).

Antibiotic	*n*	EA (%)	CA (%)	VME	ME	MiE	S	I	R
Gram-positive									
Ampicillin	14	92.9	100	0	0	0	9	0	5
Ceftaroline	1	NA[a]	100	0	0	0	1	0	0
Daptomycin	74	98.6	100	0	0	0	74	0	0
Doxycycline[b]	0	NA	NA	0	0	0	0	0	0
Erythromycin	65	86.2	86.2	6	0	3	30	2	33
Linezolid	76	100	100	0	0	0	76	0	0
TMP-SMX	63	98.4	98.4	0	1	0	62	0	1
Vancomycin	72	91.7	97.2	0	0	2	68	0	4
Gram-negative									
Amikacin	48	100	100	0	0	0	48	0	0
Ampicillin-sulbactam	38	94.7	73.7	0	2	8	26	6	6
Aztreonam[b]	0	NA	NA	0	0	0	0	0	0
Cefazolin	32	96.9	78.1	0	1	6	24	5	3
Cefepime	5	80.0	80.0	0	0	1	5	0	0
Ceftazidime	26	84.6	88.5	0	0	3	22	0	4
Ceftriaxone	26	96.2	92.3	0	0	2	21	0	5
Ciprofloxacin	49	100	98.0	0	0	1	38	4	7
Colistin[b]	0	NA	NA	0	0	0	0	0	0
Ertapenem	26	100	100	0	0	0	25	1	0
Gentamicin	48	100	97.9	0	0	1	45	0	3
Meropenem	31	100	100	0	0	0	31	0	0
Minocycline[b]	0	NA	NA	0	0	0	0	0	0
Piperacillin-tazobactam	49	98.0	91.8	0	0	4	47	1	1
Tobramycin	47	100	95.7	0	0	2	44	1	2
Overall	**790**	**96.5**[a]	**94.6**	**6**	**4**	**33**	**696**	**20**	**74**

VME = very major error (false susceptibility); ME = major error (false resistance); MiE = minor error [one system is intermediate (I) and the other is susceptible (S) or resistant (R)]; TMP-SXT = trimethoprim-sulfamethoxazole.
[a] Ceftaroline did not have an MIC result and was therefore not included in the EA calculations.
[b] Aztreonam, doxycycline, colistin, and minocycline were not tested by standard of care.

Table 4
ID and AST performance based on bottle type.

Bottle type	Number eligible (out of 237)	Number polymicrobic	Organism identification performance		Off-panel organisms % (*n*)	AST performance (all drugs combined)					Number Gram neg, Gram pos, yeast, unknown ID
			PPA	NPA		EA	CA	VME	ME	MiE	
FA Plus	150	3	137/147 (93.2%)	2220/2242 (99.0%)	5.9 (9)	502/521 (96.4%)	490/522 (93.9%)	3	3	26	104, 42, 7, 0
FN Plus	50	1	39/41 (95.1%)	748/753 (99.3%)	15.7 (8)	158/164 (96.3%)	158/164 (96.3%)	2	0	4	34, 17, 0, 0
PF Plus	37	0	30/30 (100%)	544/556 (97.8%)	18.9 (7)	101/104 (97.1%)	99/104 (95.2%)	1	1	3	11, 24, 0, 2
Total	237	4	206/218 (94.5%)	3512/3551 (98.9%)	10.0 (24)	761/789 (95.6%)	747/790 (94.6%)	6	4	33	149, 83, 7, 2

3.3. Performance by bottle type

Media components differ among the 3 bottle types used in this study (FA Plus, FN Plus, and PF Plus) and could potentially affect performance of the Accelerate Pheno™ system. AXDX performed well for each bottle type for ID and AST (Table 4). The PPA of the PF Plus bottle for identification was 100%. Very major errors, major errors, and minor errors for FA Plus, FN Plus, and PF Plus represented 6.1%, 3.7%, and 4.8%, respectively, of the total AST comparisons performed.

3.4. Time to ID and AST

Fifty-two samples were excluded from timing analysis due to errors misrepresenting the actual times to ID, including cases in which the initial Gram stain time was entered as the time to ID and cases in which the second positive bottle from a patient was loaded on the Accelerate Pheno™ system but the time for ID was entered for the first positive bottle. A final total of 185 samples were analyzed for ID timing (Table 5). The average time to ID with the SOC was 26.8 h. AXDX reduced this time to ID by an average of 24.9 ± 6.9 h ($P \leq 0.001$) and produced an ID result in 1.9 h from time of blood culture positivity.

For those samples where AXDX and SOC ID results matched, the average time difference is further broken down by organism (ID probe) in Table A.4.

For AST, the Accelerate Pheno™ system reported results an average of 7.1 h from the time of blood culture positivity. AXDX reported susceptibility data an average of 36.7 ± 18.9 h ($P \leq 0.001$) (49.3 h for Gram-positives and 26.9 h for Gram-negatives) faster than the SOC (Table A.5).

4. Discussion

Our study found the Accelerate Pheno™ system can provide rapid ID/AST results (~7 h) after a blood culture bottle flags positive. The average time to ID using our SOC methods was 26.8 h, while the Accelerate Pheno™ system reported ID in an average of 1.9 h, a reduction of 24.9 h. Similarly, the Accelerate Pheno™ system reduced the time to AST by 36.7± 18.9 h ($P \leq 0.001$). Knowing the infectious etiology earlier and choosing appropriate and targeted antibiotic therapy could facilitate de-escalation of unnecessary antibiotic coverage and lower health-associated costs for patients and hospitals (Barenfanger et al., 1999; Doern et al., 1994; Fraser et al., 2006; Galar et al., 2012). Taken together, the reduced time to ID and AST suggests that utilization of the Accelerate Pheno™ system could significantly impact patient care.

Table 5
Average time to ID and AST results for AXDX vs. SOC from time of blood culture positivity (in hours ± standard deviation) (n=185) and P value of difference.

	AXDX	SOC	Difference	*P* value
Time to ID	1.9 ± 0.0	26.8 ± 6.9	24.9 ± 6.9	≤0.001
Time to AST	7.1 ± 0.1	43.8 ± 18.8	36.7 ± 18.9	≤0.001

The AST capabilities of the Accelerate Pheno™ system produced results more than an entire day sooner than the SOC methods used in our laboratory. The time difference in the Gram-positive and Gram-negative results using the MicroScan® Walkaway® system reflects the difference in time the AST panels must incubate before reading.

Unlike previous studies of the Accelerate Pheno™ system (Charnot-Katsikas et al., 2018; Marschal et al., 2017), we used bioMérieux resin bottles. These bottles did not interfere with Accelerate Pheno™ system test performance despite different media formulations for each of the 3 BacT/ALERT bottle types. This observation suggests that the system may be applied to adult and pediatric blood cultures collected in resin-containing bottles.

In addition to the benefits of this system listed above, we observed a few drawbacks/limitations in the pre–FDA-cleared version of the software that have since been addressed in the currently marketed product. In this study, there were 13 cases (approximately 5% of samples) where the BacT/ALERT® system flagged a bottle as positive but there were too few cells for Accelerate Pheno™ system analysis (described as ID failure). Paradoxically, as blood culture detection algorithms improve and require less and less organism burden, performance of technologies used downstream (including ID and AST) might be unintentionally negatively impacted. A potential concern when considering implementation of the Accelerate Pheno™ system is the financial impact of wasted cartridges and technologist time in cases of ID failure. However, the ID failure rate was addressed in a post-FDA-clearance software update. Cases where no ID results are reported at all were reduced to 0.1% (Pancholi et al., 2018). In the case of an ID failure, a report would be generated indicating the error approximately 90 min after loading and the run would end at that time. For AST which requires ~7 h to complete, AST failures could have a higher impact

Another issue observed in the pre–FDA-cleared version of software run in this study was low PPA regarding *Pseudomonas aeruginosa* identification. Only 3 of the 4 *Pseudomonas aeruginosa* isolates encountered in this study were reported by Accelerate Pheno™ system. With such a small number of this organism detected in our study, it is difficult to predict whether this trend could be extrapolated to larger numbers of *P. aeruginosa* in our patient population. Since this study concluded, a software update which was included in the FDA-cleared product improved overall *P. aeruginosa* PPA (Accelerate Diagnostics, 2018). With this software update, another larger study reported a PPA of 98.3% and NPA of 99.8% for *Pseudomonas aeruginosa*, where 57 of 58 isolates were detected by AXDX (Pancholi et al., 2018). This suggests that, in other patient populations, the updated AXDX software performs well for *P. aeruginosa*, an organism that can carry many resistance mechanisms and is of clinical importance.

Of the 7 very major errors reported by the Accelerate Pheno™ system, 1 occurred when testing cefoxitin resistance by *S. aureus*. For *S. aureus* tested with cefoxitin, there was only 1 VME out of 18 *S. aureus* reported resistant by SOC. A multicenter study with a larger number of samples suggests that cefoxitin test results provided by

AXDX can replace traditional phenotypic testing (Pancholi et al., 2018). The remaining 6 VMEs occurred with erythromycin drug susceptibility testing. We did not detect whether erythromycin resistance was constitutive or inducible for these organisms, which might have affected the VMEs observed. While these 6 false susceptibility results for erythromycin resulted in an overall 8.1% VME rate, the software update submitted to the FDA and now part of the FDA-cleared IVD Accelerate Pheno™ system corrects for these erroneous calls. Additional postmarket comparative studies should help show whether software updates have indeed reduced the VME and ME rates associated with the Accelerate Pheno™ system. If increased rates of resistance could lead to increased error rates, 1 limitation of this study is the overall low number of resistant isolates to all of the drugs tested.

4.1. Impact on detection of MDRO

We had 3 cases of MDRO organisms during our study but no carbapenem resistance. Other studies have suggested that the Accelerate Pheno™ system could provide the AST profile of MDRO significantly faster than traditional SOC AST methods (Marschal et al., 2017; Pantel et al., 2018). The overall amount of time saved in reporting AST using the Accelerate Pheno™ system that was observed in this study supports this possibility. The Accelerate Pheno™ system reported the phenotypic susceptibility of 3 resistant Gram-negative rods in this study (Table 5), an increasing problem in hospitals from the point of view of emerging resistance and antimicrobial stewardship (Rhodes et al., 2017). Of note, there were 2 cases of resistance detected by the Accelerate Pheno™ system that highlighted the potential utility of rapid phenotypic susceptibility testing: 1 case of a potential de-repressed AmpC β-lactamase and 1 case of piperacillin-tazobactam resistance, both reported within 7 h of bottle positivity.

Detecting carbapenemase, ESBL, or AmpC production could enhance patient care by initiating correct antibiotic therapy more than a day sooner and de-escalating inappropriate therapy.

5. Conclusions

Utilizing a pre–FDA-cleared version of the Accelerate Pheno™ system, we were able to reduce the average time to identification by 24.9 ± 6.9 h ($P \leq 0.001$) and the average time to AST by 36.7 ± 18.9 h ($P \leq 0.001$) compared with our SOC. AXDX was capable of generating the ID and AST report of blood pathogens in 1.9 and 7.1 h post positive blood culture, respectively, compared with 26.8 h required for SOC ID and greater than 40 h for AST. PPA and NPA of AXDX ID were 94.5% and 98.9%. Equivalent performance for all 3 BacT/ALERT bottle types was described for the first time in this study.

Importantly, the Accelerate Pheno™ system can detect phenotypic resistance to multiple antibiotics independent of mechanism (AmpC, porin changes, ESBL, and efflux pumps) that is not detected by current commercial molecular-based genetic determinants. The high PPA and NPA of this new technology, along with fast reporting of ID and AST, could significantly impact patient care and antimicrobial stewardship.

Conflicts of interest

None declared for all authors.

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Appendix A

Table A.1
Study exclusions

Exclusions	(*n*)	%
Total samples	261	--
AXDX technical failure	6	2.3%
AXDX run >8 h post positivity	5	1.9%
AXDX ID failure	13	5.0%
Evaluable runs for ID analysis	**237**	**--**
AXDX partial AST failure	5	1.9%
AXDX total AST failure	13	5.0%

Table A.2
Testing of discrepant AST results

Organism	Antibiotic	Error	Adjudication	AXDX		SOC		BMD	
				MIC	SIR	MIC	SIR	MIC	SIR
E. faecium	Daptomycin	EA	AXDX	≤1	S	4	S	2	S
E. faecium	Vancomycin	EA	SOC	8	I	≥32	R	>64	R
E. faecium	Daptomycin	EA	AXDX	≤1	S	4	S	2	S
E. faecium	Vancomycin	EA	SOC	8	I	≥32	R	>64	R
E. faecium	Daptomycin	EA	AXDX	≤1	S	4	S	2	S
E. coli	Cefazolin	VME	AXDX	≤2	S	≥8	R	1	S
E. coli	Ciprofloxacin	VME	AXDX	≤1	S	≥4	R	0.125	S
E. coli	Tobramycin	VME	AXDX	≤4	S	≥16	R	0.5	S
K. oxytoca	Ampicillin Sulbactam	ME	SOC	≥32	R	≤8	S	8	S

Table A.2 *(continued)*

Organism	Antibiotic	Error	Adjudication	AXDX		SOC		BMD	
				MIC	SIR	MIC	SIR	MIC	SIR
K. oxytoca	Cefazolin	ME	Neither	≥8	R	≤2	S	4	I
K. oxytoca	Ampicillin-Sulbactam	ME	SOC	≥32	R	8	S	8	S
MRSA	Vancomycin	EA	AXDX	≤0.5	S	2	S	0.5	S
P. aeruginosa	Cefepime	ME	SOC	16	R	≤4	S	2	S
P. aeruginosa	Ceftazidime	ME	SOC	16	R	≤2	S	2	S
S. marcescens	Ciprofloxacin	VME	AXDX	≤1	S	≥4	R	0.125	S
S. marcescens	Gentamicin	VME	AXDX	≤2	S	≥16	R	0.5	S
S. marcescens	Meropenem	VME	AXDX	≤1	S	8	R	0.031	S
S. aureus	Erythromycin	VME	SOC	0.5	S	≥8	R	≥16	R
S. aureus	Erythromycin	VME	SOC	≤0.5	S	≥8	R	16	R
S. aureus	Daptomycin	VME	Unresolved	≤0.5	S	≥2	R	1	I
S. aureus	Erythromycin	ME	AXDX	≥8.0	R	0.5	S	16	R
S. aureus	Vancomycin	EA	AXDX	≤0.5	S	2	S	1	S
S. aureus	Vancomycin	EA	AXDX	≤0.5	S	2	S	1	S
S. aureus	Erythromycin	VME	SOC	0.5	S	≥8	R	16	R
S. aureus	Erythromycin	VME	SOC	0.5	S	≥8	R	16	R
S. hominis	Erythromycin	VME	AXDX	0.5	S	≥8	R	0.125	S
S. lugdunensis	TMP-SMX	ME	SOC	4	R	≤0.5	S	0.5	S
S. lugdunensis	Erythromycin	VME	AXDX	0.5	S	≥8	R	0.125	S
S. lugdunensis	Erythromycin	VME	SOC	0.5	S	≥8	R	16	R

AXDX = Accelerate Pheno™ system; EA = essential agreement; ME = major error (false resistance); SOC = standard of care; VME = very major error (false susceptibility), TMP-SMX = trimethoprim-sulfamethoxazole; BMD= broth microdilution.

Table A.3
Resistance of 3 isolates with multidrug resistance: *E. coli*, *S. marcescens*, and *Enterobacter* spp.

Antimicrobial name	AXDX RR	SOC RR	Bkpt low	Bkpt high	AXDX MIC	SOC MIC	AXDX SIR	SOC SIR
Escherichia coli								
Cefazolin	0.5-16	2-8	2	8	≥8.0	≥8.0	R	R
Cefepime[a]	1-32	4-32	2	16	≥32	≥32.0	N/A	N/A
Ceftazidime	2-32	1-32	4	16	≥32	≥32.0	R	R
Ceftriaxone	0.25-8	1-64	1	4	≥8	≥8.0	R	R
Ampicillin/sulbactam	2-64	8-32	8	32	≥32.0	16	R	I
Piperacillin/tazobactam	4-256	16-128	16	128	128	32	R	I
Ertapenem	0.125-4	0.5-4	0.5	2	≤0.5	≤0.5	S	S
Meropenem	0.25-8	1-16	1	4	≤1.0	≤1	S	S
Ciprofloxacin	0.25-8	1-4	1	4	≥4.0	≥4.0	R	R
Amikacin	4-128	16-64	16	64	≤16.0	≤16	S	S
Gentamicin	1-32	2-16	4	16	≤2.0	≤2	S	S
Tobramycin	1-32	4-16	4	16	≤4.0	≤4	S	S
Serratia marcescens								
Cefepime	1-32	4-32	2	16	4	≤4	N/A	N/A
Ceftazidime	1-32	1-32	4	16	≥32	≥32.0	R	R
Ceftriaxone	0.5-8	1-64	1	4	≥8	8	R	R
Piperacillin/tazobactam	4-256	16-128	16	128	32	≤16	I	S
Ertapenem	0.125-4	0.5-4	0.5	2	≤0.5	≤0.5	S	S
Meropenem	0.25-8	1-16	1	4	≤1.0	≤1	S	S
Ciprofloxacin	0.25-8	1-4	1	4	2	2	I	I
Amikacin	4-128	16-64	16	64	≤16.0	≤16	S	S
Gentamicin	1-32	2-16	4	16	16	≥16.0	R	R
Tobramycin	1-32	4-16	4	16	16	≥16.0	R	R
Enterobacter spp.								
Cefepime	1-32	4-32	2	16	16	≤4	N/A	N/A
Ceftazidime	2-32	1-32	4	16	≥32	≥32.0	R	R
Ceftriaxone	0.25-8	1-64	1	4	4	≥8.0	R	R
Piperacillin/tazobactam	4-256	16-128	16	128	128	≥128.0	R	R
Ertapenem	0.125-4	0.5-4	0.5	2	1	1	I	I
Meropenem	0.5-8	1-16	1	4	≤1.0	≤1	S	S
Ciprofloxacin	0.5-8	1-4	1	4	≤1.0	≤1	S	S
Amikacin	4-128	16-64	16	64	≤16.0	≤16	S	S
Gentamicin	1-32	2-16	4	16	≤2.0	≤2	S	S
Tobramycin	1-32	4-16	4	16	≤4.0	≤4	S	S

AXDX = Accelerate Pheno™ system; RR = reportable range; SOC = standard of care.
[a] For cefepime, the lower end of the SOC reportable range was in the middle of the CLSI (2016) breakpoints. Therefore, CA was not assessed.

Table A.4
Average difference in time to ID (AXDX vs. SOC) for samples where AXDX and SOC reported the same ID (n=172)

Organism	Probe	Count (n)	Average time difference SOC minus AXDX (h)
Gram-positive			
Staphylococcus aureus	SAU	47	25.1
Coagulase-negative *Staphylococcus* spp.	CNS	50	23.1
Staphylococcus lugdunensis	SLU	5	26.6
Enterococcus faecalis	EFS	9	22.2
Enterococcus faecium	EFM	5	27.7
Streptococcus spp.	STR	10	22.2
	Total	**126**	**24.0**
Gram-negative			
Escherichia coli	ECO	22	24.4
Klebsiella spp.	KLE	7	29.5
Enterobacter spp.	ENT	2	39.0
Proteus spp.	PRO	1	28.3
Serratia marcescens	SMA	3	24.6
Pseudomonas aeruginosa	PAE	4	31.8
	Total	**39**	**26.9**
Yeast			
Candida albicans	CAL	2	32.1
Candida glabrata	CGL	5	23.7
	Total	**7**	**26.1**
	Overall Total	**172**	**24.8**

Table A.5
Average difference in time to AST (AXDX vs. SOC)

Antimicrobial	Count (n)	Average time difference SOC minus AXDX (h)
Gram-positive		
Ampicillin	6	54.3
Daptomycin	33	49.7
Erythromycin	29	48.4
Linezolid	35	49.4
Trimethoprim-sulfamethoxazole	27	46.7
Vancomycin	38	48.9
Cefoxitin	15	55.4
MLSb	1	17.1
Total	**184**	**49.3**
Gram-negative		
Cefazolin	14	26.9
Ceftriaxone	15	29.2
Ceftazidime	18	26.1
Cefepime	15	22.9
Ampicillin-sulbactam	18	25.9
Piperacillin-tazobactam	23	26.7
Ertapenem	20	25.4
Meropenem	21	28.3
Amikacin	23	26.7
Gentamicin	23	28.3
Tobramycin	22	26.2
Ciprofloxacin	23	28.9
Total	**235**	**26.9**
Overall total	**419**	**36.7**

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