Prospective Evaluation of Accelerate Pheno™ System (AXDX) Version 1.1 for Reducing Turn-Around-Time (TAT) in Identification/Antimicrobial Susceptibility Testing (ID/AST) of Gram-Negative Bacilli (GNB) from Positive Blood Cultures (+BC) using AXDX PhenoTest™ BC Kits

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UPDATED ABSTRACT

Background: This study evaluated AXDX (uses FISH/real-time microscopy to obtain ID/AST direct from +BC in <7h) for accuracy against reference mass spectrometry (MS, VITEK® MS, bioMérieux) and broth microdilution (BMD), respectively. AXDX TAT was timed against current reporting for ID using MS and VITEK®2 (VT2, bioMérieux) for AST (VT2 data not presented).

Methods: Blood from 171 semi-consecutive +BC tested in BacT/Alert® (bioMérieux) with GNB on Gram were tested in AXDX. Susceptible/intermediate/resistant (S/I/R) interpretations were as per CLSI-M100-S27 for cefazolin (CFZ), ceftriaxone (CRO), ceftazidime (CAZ), piperacillin/tazobactam (TZP), ertapenem (ETP), meropenem (MEM), ciprofloxacin (CIP), gentamicin (GM), tobramycin (TOB), and amikacin (AN). ID and AST results were combined across GNB-genera for assessment as per Cumitech 31A for ≥90% agreements [identificatoin, essential (EA); categorical (CA)] and errors [very major (VME) <3%; combined major/minor (ME/mE) <7%].

Results: 143 (83.6%) AXDX results were evaluable (ID-agreed/AST-reported) for 78 *Escherichia coli*, 27 *Klebsiella* species, 17 *Pseudomonas aeruginosa*, 9 *Serratia marcescens*, 5 *Enterobacter cloacae*, 3 *Proteus mirabilis*, 2 *Acinetobacter baumannii* and 2 *Citrobacter freundii* as tabulated below. Limits were exceeded for underlined values but 95% confidence intervals (CI) overlapped acceptable limits except in values marked with asterisks [EA (CAZ: 68.9-82.9), ME/mE (CFZ: 5.1-16.8; CAZ: 9.9-21.8; TZP: 9.7-21.5)]. VME were not evaluable (NE) for TZP, ETP, MEM, or AN due to insufficient GNB resistant to these agents.

Evaluable (n=143)	CFZ	CRO	CAZ	TZP	ETP	MEM	CIP	GEN	TOB	AMK
AXDX-AST (no)	105	124	141	143	124	143	143	141	140	141
BMD-S (no.)	75	106	125	136	124	142	119	128	125	141
BMD-I (no.)	8	1	0	6	0	1	1	1	12	0
BMD-R (no.)	22	17	16	1	0	0	23	12	3	0
%EA	96.2	89.5	76.6*	85.3	99.2	94.4	96.5	92.2	93.6	99.3
%CA	89.5	91.9	85.1	85.3	99.2	97.9	98.6	98.6	95.7	100
%VME	4.6	5.9	0	1 NE	NE	NE	0	0	0	NE
%ME/mE	9.5*	7.3	14.9*	14.7*	0.8	2.1	1.4	0.7	4.3	0

•ID/AST TAT means (ranges) for AXDX were 1:21h (1:19-1:24h)/6:38h (6:30-6:59h) compared to MS/VT2 of 8:49h (2:49-76:48h)/38:23h (19:44-64:22h), respectively; paired t-tests, P<0.0001/P<0.0001; difference of means: 8:17h (95%CI:6:55-9:38h)/28:41h (95%CI:26:53-30:29h)].

•**Conclusions:** These data suggest AXDX will significantly reduce ID/AST TAT in GNB bacteremia (ID: 8:49h to 1:21h; AST: 38:23h to 6:38h) with potential to significantly improve patient outcomes. Acceptable accuracy was achieved for ID and AST for most agents. A limitation was the lack of GNB causing bacteremia with resistance to AN, TZP, ETP or MEM in during evaluation.

INTRODUCTION

Rapid accurate reporting of organism identifications (ID) with corresponding antimicrobial susceptibility test (AST) profiles from positive blood cultures (BC) is integral to reducing morbidity and mortality in acutely ill patients presenting with severe bacteremia. While many clinical microbiology laboratories have in recent years significantly improved ID turn-around-time (TAT) by implementing MALDI-TOF mass spectrometry (MS), AST TAT is comparatively slow as most continue to rely on conventional algorithms using culture-based or automated AST methods that have not been modified for decades where results may only become available ~24-72h after the Gram stain report.

To address this issue, Accelerate Diagnostics (AXDX, Tucson, Arizona, US) recently introduced a novel rapid (<7h) low-complexity ID/AST platform for testing blood directly from newly-positive BC bottles. The AXDX Pheno™ system requires that a sample of blood culture be transferred from the BC bottle to the AXDX PhenoTest™ BC kit sample vial, and for this vial to be clipped into a reagent cartridge, which together with a cassette, is inserted into the instrument for testing. After processing to remove blood products, all bacteria (or yeast) present are stained with Acridine Orange to enable a total organism count and a mono-microbial call. This is followed by staining with a limited panel of fluorescent *in situ* hybridization (FISH) probes that can detect the more common organisms implicated in septicaemias. Once a bacterial ID has been achieved (~1h:20m), the remaining bacterial cells are distributed through the cassette to be immobilized on thin layers of agarose containing antimicrobials appropriate to the specific organism ID. Using precise coordinates, each immobilized bacterial cell is monitored for its ability to grow (or not) while in the presence of each particular antibiotic. Growth is measured at the cellular level using automated microscopy with image capture over 5h that tracks the progress of each individual cell. Once image capture has been completed, the cumulative cellular behavior of the test organism during its 5h exposure to each antibiotic, is compared graphically to AXDX's predictive database containing previously amassed data correlating to behavior of numerous similar organisms with susceptible (S), intermediate (I) and resistant (R) phenotypes to each agent on the panel. AXDX software proceeds to predict S/I/R categorical results as well as the specific minimum inhibitory concentration (MIC) for each antibiotic. Thus, ID/AST with MIC may be reported <7h after Gram stain so long as a single organism hybridizes to a genus/species-specific probe on the AXDX PhenoTest™ BC kit ID menu.

This study evaluated the AXDX system ID/AST accuracy and TAT prospectively. Non-duplicate BC with Gram-negative bacilli (GNB) on Gram stain from aerobic BC were enrolled in a semi-consecutive manner. Samples of blood were drawn for ID/AST in AXDX <8h of BC positivity. As comparator, isolates were subjected to gold standard MS-ID and CLSI broth microdilution (BMD) for AST.

METHODS

- 171 semi-consecutive non-duplicate BC that flagged positive after incubation in the BacT/Alert® 3D system (bioMérieux) were enrolled into the Accelerate prospective study when GNB had been seen on Gram stain (poly-microbial BC mostly avoided).
- BacT/Alert® FA Plus O2 bottles were mostly used to avoid anaerobes/fastidious GNB (FISH probes not on AXDX ID menu).
- As per AXDX, blood from BC bottles was transferred <8h of flag-time to sample vials supplied in AXDX PhenoTest™ BC kits. Blood was also cultured to 5% Columbia Sheep Blood agar (BA, Oxoid). Vials were clipped into reagent cartridges, which were inserted along with cassettes into AXDX Pheno™ system modules for ID/AST determinations.
- Times were recorded for: BC-positive in BacT/Alert® system, Lab Gram report (from LIS), AXDX set-up, AXDX ID/AST TAT, Lab ID report (from LIS; ID done by MALDI-TOF MS from short incubation cultures using bioMérieux VITEK®MS Plus), Lab standard-of-care AST report (from LIS; done using AST-N213 cards in VITEK®2), and AST-N213 result (from VITEK®2).
- AXDX ID were compared to Lab MS ID, but as Vitek®2 is not a reference method, AXDX AST were compared to CLSI broth microdilution (BMD, AXDX) AST using from BA purity plates.
- Susceptible/intermediate/resistant (S/I/R) interpretations from AXDX and BMD were as per CLSI-M100-S27 for cefazolin (CFZ), ceftriaxone (CRO), ceftazidime (CAZ), piperacillin/tazobactam (TZP), ertapenem (ETP), meropenem (MEM), ciprofloxacin (CIP), gentamicin (GM), tobramycin (TOB), and amikacin (AN).
- For AXDX vs. MS/BMD performances, ID were combined across GNB-genera, and AST were assessed as per Cumitech 31A, for ≥90% agreements [essential (EA); categorical (CA)] and errors [very major (VME) <3%; combined major/minor (ME/mE) <7%].

RESULTS

Table 1. Accelerate (AXDX) PhenoTest™ BC kit identifications (ID) from AXDX Pheno™ system after direct from BacT/Alert® bottle testing of 171 newly-positive blood cultures resulting in 143 evaluable AST results [correct ID w/AST; No. (%) 95%CI]

Mono-microbial bloods w/ on-AXDX menu GNB (No.) (n=146)	AXDX ID correct (n=142)	AXDX ID correct w/AST (n=140)	AXDX Pheno™ ID result details (w/AST or NO AST)
Escherichia coli (75)	75 (100) 94.2-100	75 (100) 94.2-100	70 *E. coli* (w/AST) 3 *E. coli* (w/AST) w/false *C. glabrata* (NO AST) 1 *E. coli* (w/AST) w/false CNST (NO AST) 1 *E. coli* (w/AST) w/false *E. faecalis* (w/AST vanco-R)
Klebsiella pneumoniae (28)	26 (92.9) 76.3-99.1	25 (96.2) 79.6->99.9	19 *Klebsiella* spp (w/AST) 3 *Klebsiella* spp (w/AST) (*Enterobacter* indeterminate) 2 *Klebsiella* spp (w/AST) (1 CNST/1 *S. lug* both indeterminate) 1 *Klebsiella* spp (w/AST) w/false *P. aeruginosa* (NO AST) 1 *Klebsiella* spp (NO AST) w/false *S. aureus*/CNST (NO AST) 2 with NO ID (NO AST)
Pseudomonas aeruginosa (19)	18 (94.7) 73.5->99.9	17 (94.4) 72.4->99.9	16 *P. aeruginosa* (w/AST) 1 *P. aeruginosa* (w/AST) (*S. aureus*/CNST indeterminate) 1 *P. aeruginosa* (NO AST – AXDX Pheno™ analysis failure) 1 with NO ID (NO AST)
Serratia marcescens (9)	9 (100) 65.5-100	9 (100) 65.5-100	8 *S. marcescens* (w/AST) 1 *S. marcescens* (w/AST) w/false CNST (w/AST)
Enterobacter cloacae (6)	5 (83.3) 41.8-98.9	5 (100) 51.1-100	4 *Enterobacter* spp (w/AST) 1 *Enterobacter* spp (w/AST) (1 w/true CNST missed) 1 with NO ID (NO AST) but w/false CNST (NO AST)
Proteus mirabilis (3)	3 (100)	3 (100)	3 *Proteus* spp (w/AST)
Klebsiella oxytoca (2)	2 (100)	2 (100)	2 *Klebsiella* spp (w/AST)
Citrobacter freundii complex (2)	2 (100)	2 (100)	1 *Citrobacter* spp (w/AST) 1 *Citrobacter* spp (w/AST) (1 *Enterobacter* spp indeterminate)
Acinetobacter baumannii (2)	2 (100)	2 (100)	2 *A. baumannii* (w/AST)
Poly-microbial bloods w/ on-AXDX menu GNB (No.) (n=5)	**AXDX ID correct (n=4)**	**AXDX ID correct w/AST (n=3 1 spp.)**	**AXDX Pheno™ ID result details (w/AST or NO AST)**
E. coli/*K. pneumonaie* (4)	1/1 3/0	0/0 3/0	1 *E. coli* and 1 *Klebsiella* spp (both NO AST) 3 *E. coli* (w/AST) but 3 *Klebsiella* missed NO ID (NO AST)
K. pneumonaie/*K. oxytoca* (1)	0/0	0/0	1 with NO ID either species (NO AST)
Mono-microbial bloods w/ off-AXDX menu GNB (No.) (n=20)	**AXDX ID Incorrect**	**AXDX ID Incorrect w/AST (n=2)**	**AXDX Pheno™ ID result details (w/AST or NO AST)**
Stenotrophomonas maltophila (7)	0	0	ALL 17 ID reported "NEGATIVE" (NO AST generated)
Acinetobacter not baumannii (2)	0	0	
Rhizobium radiobacter (2)	0	0	
Sphingomonas paucimobilus (2)	0	0	
Achromobacter xylosoxidans (1)	0	0	
Brevundimonas diminuta (1)	0	0	
Hafnia alvei (1)	0	0	
Pseudomonas oryzihabitans (1)	0	0	
Acinetobacter lwoffii (2)	2	1	1 w/false *E. coli* ID (w/AST)
		0	1 w/false *C. glabrata* ID (NO AST)
Cronobacter sakazakii (1)	1	1	1 w/false *Klebsiella* spp ID (w/AST)

RESULTS cont'd

In total, of 171 BC, 146 (85.4%) had GNB with correct AXDX ID and 143 (83.6%) had evaluable AXDX AST while 10 (5.8%) had false positive AXDX ID, of which 3 (1.7%) had AST.

Table 1 summarizes AXDX ID results from 171 BC BacT/Alert® bottles. Of these, 146 (85.4%) BC grew single GNB (mono-microbial) identifiable by AXDX (on-AXDX menu), 5 (2.9%) BC grew two on-AXDX menu species (poly-microbial), and 20 (11.7%) BC grew GNB with no species probe on AXDX's ID menu (off-AXDX menu).

- Of 146 on-menu single-spp. GNB, 142 (97.3%; 95%CI:92.9-99.2) had correct AXDX ID, but 4 (2.7%) were negative.
- Of 5 polymicrobial on-menu GNB, 1 BC with *E. coli*/*Klebsiella* had AXDX correct ID/no AST for both GNB, and in 3 other *E. coli*/*Klebsiella* mixes, AXDX produced ID/AST for *E. coli* only, while in 1 *K. pneumonaie*/*K. oxytoca* mix, neither GNB was detected by AXDX.
- Of 20 off-menu GNB, 17 (85%) were AXDX ID-negative, but 3 (15%) were misidentified, 2 (10%) with AST resulted.

Table 2 summarizes AXDX AST from 143 (83.6%) BC (140 ID from mono-microbial BC, 3 ID from polymicrobial BC).

- Values shaded in grey indicates too few resistant GNB tested to assess AXDX VME rates for that antibiotic
- Values shaded in green met Cumitech limits
- Values shaded in yellow overlapped 95% CI ranges for acceptable limits
- Values shaded in red failed to meet or overlap 95%CI ranges for acceptable limits

- The bulk of AXDX AST errors were minor errors associated with *P. aeruginosa* and CAZ [12/33 (36.4%) EA; 13/21 (61.9) mE; 1/3 (33.3) ME] and TZP [11/21 (52.4%) mE] while remaining errors were associated with diverse GNB spp.

ID/AST TAT means (ranges) for AXDX were 1:21h (1:19-1:24h)/6:38h (6:30-6:59h) compared to MS/VT2 of 8:49h (2:49-76:48h)/38:23h (19:44-64:22h), respectively; paired t-tests, P<0.0001/P<0.0001; difference of means: 8:17h (95%CI:6:55-9:38h)/28:41h (95%CI:26:53-30:29h)].

Table 2. Summary of performance of Accelerate Pheno™ system for 143 prospective Gram-negative bacilli (genera combined) with evaluable ID/AST obtained <7h directly from positive blood cultures where ID/AST compared to VITEK ®MS Plus MALDI-TOF ID and CLSI reference broth microdilution (BMD) AST respectively

Antibiotic	CFZ	CRO	CAZ	TZP	ETP	MEM	CIP	GEN	TOB	AMK
No. AXDX-AST	105	124	141	143	124	143	143	141	140	141
No. S by BMD	75	106	125	136	124	142	119	128	125	141
No. I by BMD	8	1	0	6	0	1	1	1	12	0
No. R by BMD	22	17	16	1	0	0	23	12	3	0
AXDX EA No. (%) 95% CI	101 (96.2) 90.3-98.8	111 (89.5) 82.8-93.9	108 (76.6) 68.9-82.9	122 (85.3) 78.5-90.3	123 (99.2) 95.1->99.9	135 (94.4) 89.2-97.3	138 (96.5) 91.8-98.7	130 (92.2) 86.4-95.7	131 (93.6) 88.1-96.7	140 (99.3) 95.7->99.9
AXDX CA No. (%) 95% CI	94 (89.5) 82.1-94.2	114 (91.9) 85.6-95.7	120 (85.1) 78.2-90.1	122 (85.3) 78.5-90.3	123 (99.2) 95.1->99.9	140 (97.9) 93.7-99.6	141 (98.6) 94.7-99.9	139 (98.6) 94.7-99.9	134 (95.7) 90.8-98.2	141 (100) 96.8-100
AXDX VME No. (%) 95% CI	1 (4.6) <0.01-23.5	1 (5.9) <0.01-28.9	0 (0) 0-22.7	1 NE	NE	NE	0 (0) 0-16.9	0 (0) <0.01-37.5	0 (0) 0-61.8	NE
AXDX ME No. (%) 95% CI	0 (0) 0-5.8	1 (0.9) <0.01-5.7	3 (2.1) 0.5-6.4	2 (1.4) 0.06-5.3	0 (0) 0-3.6	0 (0) 0-3.2	1 (0.8) <0.01-5.1	0 (0) 0-3.5	0 (0) 0-3.6	0 (0) 0-3.2
AXDX mE No. (%) 95% CI	10 (9.5) 5.1-16.8	8 (6.5) 3.1-12.4	18 (12.8) 8.1-19.4	19 (13.3) 8.6-19.9	1 (0.8) <0.01-4.9	3 (2.1) 0.4-6.3	1 (0.7) <0.01-4.3	1 (0.7) <0.01-4.3	6 (4.3) 1.8-9.2	0 (0) 0-3.2
AXDX ME/mE No. (%) 95% CI	10 (9.5) 5.1-16.8	9 (7.3) 3.7-13.4	21 (14.9) 9.9-21.8	21 (14.7) 9.7-21.5	1 (0.8) <0.01-4.9	3 (2.1) 0.4-6.3	2 (1.4) 0.06-5.3	1 (0.7) <0.01-4.3	6 (4.3) 1.8-9.2	0 (0) 0-3.2

CONCLUSIONS

- **These data suggest AXDX will substantially reduce ID/AST TAT in 83.6% of GNB bacteremia with potential to significantly improve patient outcomes**
- **Average microbiology laboratory ID TAT of 8:49h obtained using optimized MALDI-TOF methods with timing from Gram stain report was reduced to only 1:21h by AXDX testing**
- **Similarly, average laboratory AST TAT of 38:23h using current algorithms was reduced to 6:38h after Gram stain report. In addition, MIC results were reported together with CLSI S/I/R interpretation for all antibiotics tested.**
- **Acceptable accuracy was achieved for ID and AST for most organisms and agents; software version updates are being released to attempt to minimize BC with false ID and reduce AST errors**
- **This study's limitation was that resistance rates for AN, TZP, ETP or MEM in Toronto BC were too low to assess AXDX VME. To address this deficit, retrospective GNB selected for specific genotypes was conducted simultaneously (see IDweek poster 2038), thus both posters should be viewed simultaneously.**
- **The AXDX PhenoTest™ BC kit and the AXDX Pheno™ instrumentation were very simple to use requiring only a few minutes hands-on time to set up and report and would fit easily into any laboratory workflow.**

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