Comparison of Accelerate (AXDX) BC PhenoTest™ results from Gram-negative bacilli (GNB) Parallel-seeded to FA-Plus Blood Culture Bottles (BCB) incubated in bioMérieux BacT/Alert-3D and VIRTUO systems

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UPDATED ABSTRACT

Background: Expedited identification/antimicrobial susceptibilty testing (ID/AST) is essential for optimizing bacteremia management. Previous evaluations on BCB following BacT/Alert-3D incubation indicated AXDX's Pheno™ system (version 1.1.1) could provide accurate ID/AST results. As VIRTUO's closed/automated incubation is replacing 3D, this study aimed to prove AXDX-ID/AST-result equivalency following parallel-SBCB-incubations in VIRTUO and 3D systems.

Materials/methods: 74 GNB (61 *Enterobacteriaceae*, 7 *Acinetobacter baumannii*, 6 *Pseudomonas aeruginosa*; 44 (59.5%) CPO: 12 KPC, 10 NDM1/5/7, 8 OXA48-type, 4 VIM, 3 OXA23/24, 2 GES5, 2 SME, 1 VIM/GES5, 1 IMP7, 1 OXA23/NDM) were seeded to paired-BCB, simultaneously incubated in VIRTUO/3D, and parallel-tested in AXDX PhenoTM (version 1.3.1). Seeding-suspensions were also tested by broth microdilution (BMD; 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). With GNB-genera combined, VIRTUO/3D-AXDX-AST were assessed against BMD using Cumitech 31A for ≥90% agreements [essential (EA); categorical (CA)] and errors [very major (VME) <3%; combined major/minor (ME/mE) <7%].

Results: From 74 BCB-pairs, 71 (96%)/69 (93.2%) (p=0.71 NS) AXDX-IDs and 66 (89.2%)/69 (93.2%) (p=0.56 NS), AXDX-ID/ASTs were obtained from VIRTUO/3D respectively. VIRTUO/3D-AXDX-AST from 63 (85.1%) with corresponding AXDX-ID/AST is tabulated below. Underlined values exceeded limits, but 95% confidence intervals (CI) overlapped limits except in values marked with astericks [95%CI: EA (TZP-VIRTUO: 64.8-85.9; AN-VIRTUO/3D: 69-88.7/67.7-87.7); CA (TZP-VIRTUO:66.7-87.2; AN-VIRTUO/3D: 67.2-87.5/65.5-87.2); VME (ETP-3D: 3.9-26.6); ME/mE (CAZ-3D: 7.3-26.4; TZP-VIRTUO/3D: 12.7-33.2/10-29.2; AN-VIRTUO/3D: 11.3-31/11.5-32.7)].

Evaluable BCB-pairs (63)	CFZ	CRO	CAZ	TZP	ETP	MEM	CIP	GEN	TOB	AN
BCB with AXDX-ID+AST (no. VIRTUO/3D)	39/39	49/50	55/56	61/62	49/49	51/52	63/62	56/56	56/56	62/63
BMD-S (no.)	1	11	13	11	13	21	18	32	21	39
BMD-I (no.)	1	1	2	10	2	4	0	1	9	8
BMD-R (no.)	37	38	41	42	35	38	45	24	26	16
%EA	97.4/97.4	91.8/92	92.7/94.6	77.1*/83.9	89.8/87.7	88.2/84.6	96.8/95.2	91.1/89.2	96.4/94.6	80.6*/79.4*
%CA	97.4/97.4	91.8/92	85.6/87.5	78.7*/82.3	87.8/88.5	88.2/88.5	98.4/100	91.1/89.2	92.9/89.3	79*/77.8*
%VME	0/0	0/0	0/0	0/0	8.6/11.4*	6.1/8.6	0/0	0/0	0/0	0/6.3
%ME+mE	2.6/2.6	8.2/8	14.5/12.5*	21.3*/17.7*	6.1/6.1	7.8/5.8	1.6/0	8.9/10.7	7.1/10.7	19.3*/20.6*

42/44 (95.5%) CPO had evaluable AXDX-ID/AST VIRTUO/3D-pairs. While 8/8 (100%) non-CPO-*Enterobacteriaceae* were BMD-MEM=R/AXDX-MEM=R (VIRTUO+3D), only 32/34 (94.1%) CPO-*Enterobacteriaceae* were CPO by AST as 2 (1 NDM-*Proteus mirabilis*, 1 KPC-*Escherichia coli*) tested BMD-ETP=S/AXDX-ETP=S. Of 32 AST-detectable CPO-*Enterobacteriaceae* (BMD-ETP: 1->16mg/L), 3 AXDX-ETP=S (VME) were from VIRTUO+3D (KPC-*Enterobacter cloacae*, VIM-*Klebsiella oxytoca*, SME-*Serratia marcescens*) and 1 from 3D (KPC-*Citrobacter freundii*). In 2 (OXA232-*K. pneumoniae*-VIRTUO, NDM-*E. cloacae*-3D), AXDX-rules prevented ETP/MEM-related VME by ETP-suppression. Similarly, AXDX-MEM-suppression prevented VME (VIRTUO+3D) in 6/32 (18.8%) BMD-ETP=R/MEM=I/R CPO-*Enterobacteriaceae*, but it also suppressed 4/32 (12.5%) BMD-MEM=S/AXDX-MEM=S (non-VME), while 3 BMD-ETP=R/AXDX-ETP=S VME were not prevented.

Conclusions: This study found no significant differences between AXDX-ID/AST resulting from VIRTUO versus BacT/Alert-3D incubations. AXDX-rules (v1.3:1) mitigated but could not completely prevent CPO-associated VME suggesting room for further optimization of these algorithms.

INTRODUCTION

Providing expedited organism identifications (ID) and associated antimicrobial susceptibility tests (ID/AST) results is essential for optimizing management and integral to reducing morbidity and mortality in acutely ill patients presenting with severe bacteremia. Rapid ID (by ~1:20h) and AST (by ~6:30h) recently became possible using the Accelerate Diagnostics (AXDX, Tucson, Arizona, US) Pheno™ System, a low-complexity AST platform, that can provide results directly from samples drawn from positive blood cultures and tested using AXDX's PhenoTest™ BC kit. The AXDX Pheno™ system requires only that a sample of blood be transferred from the positive bottle to the AXDX PhenoTest™ BC kit sample vial, and for the 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 enabling a total organism count and a mono-microbial call. Cells are then stained with a limited panel of fluorescent *in situ* hybridization (FISH) probes to detect the more common species implicated in septicaemias. Once ID is achieved (~1:20h), the remaining cells are distributed through the cassette to be immobilized on thin agarose layers containing antimicrobials appropriate to the specific organism ID. Using precise coordinates, each immobilized bacterial cell is monitored for its ability to grow (or not) in the presence of each antibiotic. Growth is measured using automated microscopy with image capture over ~5h to track the progress of individual cells. Once image capture has been completed, the cumulative behavior of the organism during its 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 results and minimum inhibitory concentrations (MIC) for each antibiotic. Thus, ID with AST-MIC may be reported <7h of Gram strain so long as the organism present hybridizes with a specific probe on the AXDX Pheno™ BC kit ID menu. Previous evaluations of AXDX PhenoTest™ BC kit (v 1.1.1) were performed using blood with Gram-negative bacilli from BacT/Alert FA Plus bottles incubated in bioMerieux's BacT/Alert 3D System. As bioMerieux recently replaced the 3D with the BacT/Alert VIRTUO system, which provides closed more automated incubation that enables reduced time-to-positivity, this study aimed to establish AXDX-ID and AXDX-AST result equivalency by parallel-seeding blood culture bottles inoculated with a diverse array of Gram-negative bacilli followed by simultaneous incubations of bottles in VIRTUO and 3D systems, with parallel testing in the AXDX.

METHODS

- 74 GNB (61 *Enterobacteriaceae*, 7 *Acinetobacter baumannii*, 6 *Pseudomonas aeruginosa*; 44 (59.5%) CPO: 12 KPC, 10 NDM1/5/7, 8 OXA48-type, 4 VIM, 3 OXA23/24, 2 GES5, 2 SME, 1 VIM/GES5, 1 IMP7, 1 OXA23/NDM) were selected for study to determine the impact of incubations in different BacT/Alert blood culture incubating systems.

- The identity of each isolate was confirmed by MALDI-TOF using VITEK® MS Plus (bioMérieux) prior to the parallel-seeded of 75 colony forming units into each of a pair of BacT/Alert® FA Plus Aerobic blood culture bottles (bioMérieux). Bottles were supplemented immediately prior to seeding with 10mL fresh human donor blood anticoagulated with SPS (BiorelamationIVT, Westbury, NY, USA). The parallel-seeded simulated blood cultures were simultaneously incubated in bioMérieux BacT/Alert® VIRTUO and BacT/Alert® 3D systems.

- After incubation, and when flagged-positive, samples of blood were withdrawn from respective bottles and transferred into AXDX PhenoTest™ BC kit sample vials for rapid identification (ID) and antimicrobial susceptibility testing (AST) in the AXDX Pheno™ System (software version 1.3.1). As per AXDX, blood from BC bottles was transferred <8h of flag-time to sample vials. Positive blood was also cultured for single colonies to 5% Columbia Sheep Blood agar (BA, Oxoid). Filled sample vials were clipped into AXDX PhenoTest™ BC kit reagent cartridges, which were inserted along with AXDX PhenoTest™ BC kit cassettes into AXDX Pheno™ system modules for ID/AST determinations.

- Seeding-suspensions (equivalent to 0.5 McFarland STD) were also immediately tested by reference broth microdilution (BMD) with MIC endpoints interpreted as susceptible, intermediate or resistant (S, I, R) in accordance with 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).

- With GNB-genera combined, the VIRTUO versus 3D AXDX-AST were assessed against BMD using Cumitech 31A for ≥90% agreements [essential (EA); categorical (CA)] and errors [very major (VME) <3%; combined major/minor (ME/mE) <7%].

- Times were recorded (data not shown) for BC set-up time, BC-positive time in each BacT/Alert® system (for relative length of incubations), and AXDX set-up and AXDX-ID and AXDX-AST completion times (data not shown).

RESULTS

From the 74 original parallel-seeded blood culture bottle-pairs incubated in the BacT/Alert® VIRTUO and BacT/Alert 3D systems, AXDX-ID were reported from 71 (96%)/69 (93.2%) systems respectively (p=0.71 NS), while AXDX-ID plus associated AXDX-AST were reported from 66 (89.2%)/69 (93.2%) systems, respectively (p=0.56 NS), with 63 Gram-negative bacilli reporting with corresponding AXDX-ID and AST from paired bottles (Table 1).

Table 1. Summary of 63 Gram-negative bacilli species identifications with successful parallel seeding to two bioMérieux BacT/Alert® FA Plus O₂ bottles for comparison of impact of incubations in BacT/Alert VIRTUO and 3D systems on rapid ID/AST results obtained after direct-positive blood testing using AXDX's PhenoTest™ BC kits in AXDX's Pheno™ system

Original species identification	No. tested	No. correct VIRTUO-ID reporting AST	No. correct VIRTUO-ID reporting AST
Acinetobacter baumannii	7	7	7
Citrobacter freundii	1	1	1
Enterobacter cloacae	4	4	4
Escherichia coli	19	19	19
Klebsiella oxytoca	3	3	3
Klebsiella pneumoniae	17	17	17
Proteus mirabilis	3	3	3
Pseudomonas aeruginosa	6	6	6
Serratia marcescens	3	3	3
Paired BC incubated in VIRTUO and 3D BacT/Alert® systems prior to comparisons of AXDX PhenoTest™ BC kit results	63	63	63

Troubleshooting to determined the cause of non-reporting of AXDX-ID/AST was ultimately found through review of images from the failed runs to be due to micro-clots introduced into the AXDX sample vials from some lots of inappropriately processed human blood obtained specifically for seeding purposes. These AXDX runs were therefore excluded and only the 63 with paired results were included in the study. Table 1 summarizes the species identities of included isolates. Failed runs were found to be reproducible at AXDX when the same blood was obtained and tested at AXDX headquarters in Tucson, AZ.

All AXDX-ID agreed with MALDI-TOF using VITEK® MS Plus (bioMérieux).

RESULTS cont'd

For 63 (85.1%) with common corresponding AXDX-ID and AXDX-AST, Table 2 presents a detailed overview of the comparison of AXDX-AST as compared to broth microdilution MIC after VIRTUO versus 3D incubations.

- Underlined values exceeded acceptable limits, but 95% confidence intervals (CI) overlapped limits except in values marked with asterisks [95%CI: EA (TZP-VIRTUO: 64.8-85.9; AN-VIRTUO/3D: 69-88.7/67.7-87.7); CA (TZP-VIRTUO:66.7-87.2; AN-VIRTUO/3D: 67.2-87.5/65.5-87.2); VME (ETP-3D: 3.9-26.6); ME/mE (CAZ-3D: 7.3-26.4; TZP-VIRTUO/3D: 12.7-33.2/10-29.2; AN-VIRTUO/3D: 11.3-31/11.5-32.7)].

- Overall, 42/44 (95.5%) CPO had evaluable AXDX-ID/AST VIRTUO/3D-pairs.

- While 8/8 (100%) non-CPO-*Enterobacteriaceae* were BMD-MEM=R/AXDX-MEM=R (VIRTUO+3D), only 32/34 (94.1%) CPO-*Enterobacteriaceae* were CPO by AST as 2 (1 NDM-*Proteus mirabilis*, 1 KPC-*Escherichia coli*) tested BMD-ETP=S/AXDX-ETP=S.

- Of 32 AST-detectable CPO-*Enterobacteriaceae* (BMD-ETP: 1->16mg/L), 3 AXDX-ETP=S (9.4% VME) were from VIRTUO+3D (KPC-*Enterobacter cloacae*, VIM-*Klebsiella oxytoca*, SME-*Serratia marcescens*) and 1 (3.1% VME) from 3D (KPC-*Citrobacter freundii*).

- In 2 (OXA232-*K. pneumoniae*-VIRTUO, NDM-*E. cloacae*-3D), AXDX-rules prevented ETP/MEM-related VME by ETP-suppression. Similarly, AXDX-MEM-suppression prevented VME (VIRTUO+3D) in 6/32 (18.8%) BMD-ETP=R/MEM=I/R CPO-*Enterobacteriaceae*, but it also suppressed 4/32 (12.5%) BMD-MEM=S/AXDX-MEM=S (non-VME), while 3 BMD-ETP=R/AXDX-ETP=S VME were not prevented.

Table 2. Summary of performance of Accelerate Pheno™ system for 63 paired Gram-negative bacilli (genera combined) with evaluable ID/AST obtained <7h directly from positive blood cultures where AXDX results were compared to MALDI-TOF and broth microdilution (BMD) AST results

Underlined values exceeded Cumitech 31A limits, but 95% confidence intervals (CI) overlapped limits except in values marked with asterisks

Evaluable BC-pairs (63)			CFZ	CRO	CAZ	TZP	ETP	MEM	CIP	GEN	TOB	AN
BCB with AXDX-ID+AST (No. VIRTUO/3D)			39/39	49/50	55/56	61/62	49/49	51/52	63/62	56/56	56/56	62/63
BMD-S (no.)			1	11	13	11	13	21	18	32	21	39
BMD-I (no.)			1	1	2	10	2	4	0	1	9	8
BMD-R (no.)			37	38	41	42	35	38	45	24	26	16
AXDX EA	No./No.		38/39	45/46	51/53	47/52	44/43	45/44	61/59	51/50	54/53	50/50
	[%]		[97.4/97.4]	[91.8/92]	[92.7/94.6]	[77.1*/83.9]	[89.8/87.7]	[88.2/84.6]	[96.8/95.2]	[91.1/89.2]	[96.4/94.6]	[80.6*/79.4*]
	VIRTUO - 95%CI		85.6->99.9	80.3-97.3	82.3-97.6	65-85.9	77.8-96	76.3-94.9	88.5-99.8	80.3-96.5	87.2-99.7	71.1-87.6
	3D - 95%CI		85.6->99.9	80.7-97.4	84.9-98.7	72.6-91.2	75.4-94.6	72.2-92.3	86.3-98.4	84.8-98.7	67.7-87.7	
AXDX CA	No./No.		38/38	45/46	47/49	48/51	43/42	45/46	62/62	51/50	52/50	49/49
	[%]		[91.8/92]	[91.8/92]	[85.6/87.5]	[78.7*/82.3]	[87.8/85.7]	[88.2/88.5]	[98.4/100]	[91.1/89.2]	[82.5-97.7]	[79*/77.8*]
	VIRTUO - 95%CI		85.6->99.9	80.3-97.3	73.6-92.7	63.2-84.6	75.4-94.6	76.3-94.9	90.7->99.9	80.3-96.5	82.5-97.7	67.2-87.5
	3D - 95%CI		85.6->99.9	80.7-97.4	76.1-94.1	70.8-90	73-93.2	76.7-95	93-100	78.2-95.4	78.2-95.4	66-86.4
AXDX VME	No./No.		0/0	0/0	0/0	0/0	3/4	2/3	0/0	0/0	0/0	0/1
	[%]		[0/0]	[0/0]	[0/0]	[0/0]	[8.6/11.4*]	[6.1/8.6]	[0/0]	[0/0]	[0/0]	[0/6.3]
	VIRTUO - 95%CI		0-11.2	0-11.2	0-10.4	0-10	2.2-23.1	0.7-20.6	0-9.4	0-16.3	0-15.2	0-22.7
	3D - 95%CI		0-11.2	0-10.9	0-10.2	0-10	3.9-26.6	2.2-23.1	0-9.4	0-16.3	0-15.2	<0.01-30.3
AXDX ME+mE	No./No.		1/1	4/4	8/7	13/11	3/3	4/3	1/0	5/6	4/6	12/13
	[%]		[2.6/2.6]	[8.2/8]	[14.5*/12.5]	[21.3*/17.7*]	[6.1/6.1]	[7.8/5.8]	[1.6/0]	[8.9/10.7]	[7.1/10.7]	[19.3*/20.6*]
	VIRTUO - 95%CI		<0.01-14.4	2.7-19.7	7.3-26.4	12.8-33.3	1.5-17.2	2.6-19	<0.01-9.3	3.5-19.7	2.3-17.5	11.3-31
	3D - 95%CI		<0.01-14.4	2.6-19.4	5.9-23.9	10-29.2	1.5-17.2	1.4-16.3	0-7	1.5-17.2	4.7-21.8	12.3-32.3

CONCLUSIONS & DISCUSSION

- This study found no significant differences between AXDX-ID and AXDX-AST resulting from BacT/Alert® VIRTUO compared to BacT/Alert 3D system incubations, thus suggesting that either of the two systems could be used for patient blood culture incubations prior to direct rapid ID and AST using the AXDX Pheno™ system.

- As with previous evaluations, using AXDX, ID and AST for most organisms and agents was associated with a significant reduction in ID and AST TAT (ID: averaged 8:49h by optimized MALDI-TOF lab methods vs. 1:21h by AXDX; AST: averaged 38:23h by current lab AST algorithms vs. <7h by AXDX (timing data for this study not shown).

- AXDX ID agreed 100% with ID by MALDI-TOF using VITEK® MS Plus (bioMérieux). Acceptable AXDX AST performance was obtained for all antimicrobials compared to reference BMD with the exception of <90% EA and CA for TZP and AN with an associated >7% ME+mE noted with both drugs, and VME >3% associated with ETP, suggesting opportunities for further optimization of AXDX-algorithms.

- Studies documenting the impact of the use of AXDX Pheno™ system to provide rapid ID and AST directly from positive blood cultures on patient outcome are warranted.