Evaluation of a Rapid Identification and Antimicrobial Susceptibility Test System from Positive Blood Cultures

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ABSTRACT

Background: Sepsis is a significant cause of mortality, and the importance of rapid initiation of empiric therapy and subsequent tailoring of antibiotics in patient survival and preventing the emergence of resistant bacteria is well documented. Culture techniques can take up to 72 hours for definitive identification (ID) and determination of antimicrobial susceptibilities (AST). While PCR-based methodologies can provide rapid ID, they are limited in their ability to provide susceptibility data. Fast ID and AST systems like the Accelerate Pheno™ system (AXDX) dramatically reduce the time required to provide ID and susceptibility data for the organisms most commonly associated with sepsis.
Methods: AXDX was validated by Madigan Army Medical Center. 51 isolates (15 Gram-positive, 31 Gram-negative, 2 yeast and 3 off-panel) were run using AXDX and compared to the current laboratory test method (ID: FilmArray Blood Culture Identification panel, AST: VITEK® 2 system). Validation included both seeded (n=36) and patient specimens (n=17). Following AXDX validation, an additional 30 patient specimens (10 Gram-positive, 18 Gram-negative, 1 yeast, and 2 off-panel) were prospectively compared to the current methods (CM) as described.
Results: In the validation study, the positive percent agreement (PPA) and negative percent agreement (NPA) were 91.8% and 99.7%, respectively, for identification. During AST testing, two very major errors (both Gram-negative) and 12 minor errors were identified (1 Gram-positive and 11 Gram-negative). There was 100% concordance for the detection of methicillin resistance in Staphylococcus aureus (n=3) and coagulase-negative staphylococci (n=1) between AXDX and CM. From the time the blood culture was positive, the average time to ID was 2.0 hours and AST was 7.2 hours for AXDX, a potential reduction of 43.1 hours for ID and AST over CM. During the prospective study, the PPA and NPA were 80.6% and 99.0%, respectively. Two major errors were detected, both *Klebsiella oxytoca* that were ampicillin-sulbactam resistant by AXDX, but susceptible by the VITEK® 2 system. There were 5 minor errors. There was 100% concordance between methods for the cefoxitin (n=6) and inducible clindamycin screens (n=2).
Conclusion: AXDX has dramatically reduced blood culture turnaround times. There were some discordant identifications. As a result, we continue to verify the AXDX results with a second method. While the technology is promising, there are some technical issues that need to be resolved.

INTRODUCTION

- Sepsis remains a leading cause of death in the United States accounting for over 40,000 deaths in 2016
- The impact of timely, appropriate antibiotic therapy on patient survival has been well established
  - Survival decreases by about 7.6% with each hour of delay
  - Studies suggest median time to effective therapy is about 6 hrs
- Empiric therapy can guide initial treatment, however therapy should be narrowed, or may be incorrect
  - De-escalation from broad spectrum agents
  - Elimination of unneeded antimicrobials
- Traditional culture methods for identification and antimicrobial susceptibility testing can take in excess of 72 hrs
- Rapid ID and AST systems like the Accelerate Pheno have reduced the time required for ID and AST to approximately 8 hrs

MATERIALS AND METHODS

Part One

- Verification study performed following the procedures as described in the manufacturers' package insert
- Three types of specimens used, total of 52 specimens
  - Remnant positive patient specimens (n=18)
  - Challenge isolates provided by Accelerate (n=13)
  - Seeded specimens from MAMC frozen stocks (n=21)
    - Subculture (Challenge or Frozen Stocks)
    - Check purity, prepare 0.5 McFarland in sterile saline
    - Three step 1:1000 dilution
    - Inoculate 1 ml of final dilution (10-100 cfu/ml) into a blood culture bottle
  - Positive blood culture notification (BacTec Fx)
  - Perform Gram stain
  - Load on the Accelerate following manufacturer's guidance

Part Two

- Post verification
- Patient specimens tested using the Accelerate Pheno BC over an approximate three month period (n=30)
- Compared to the current laboratory method
  - Gram stain
  - Blood Culture ID Panel multiplex PCR (BioFire)
  - MALDI-ToF (Vitek-MS)
  - Susceptibility Testing (Vitek 2)

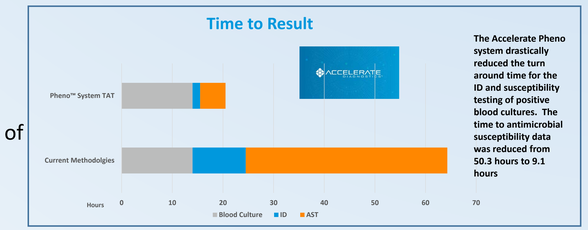
WORKFlOW

*Temporary Process

If	Then
Patient is admitted	Perform Accelerate Pheno-Test BC
Patient is not admitted	Do not perform Accelerate Pheno-Test BC
An Infectious Disease Doctor requests	Perform Accelerate Pheno-Test BC if request is possible
Organism Requirements	
Organism Gram Stain is GPR, GNC, GNDC, or Yeast	Do not perform Accelerate Pheno-Test BC
Organism Gram Stain is GNR or GNCB	Perform Accelerate Pheno-Test BC
Organism Gram Stain is GPC	Perform Biofire BCID*
Biofire BCID result *is S. aureus, Enterococcus*	Perform Accelerate Pheno-Test BC
Biofire BCID result is negative (e.g. CoNS)	Consult Microbiology leadership

GPR – Gram Positive Rods
GNC – Gram Negative Cocci
GNDC- Gram Negative Diplococci
GNCB – Gram Negative Coccobacilli
GPC Gram Positive Cocci

*** Future proposed workflow**



The Accelerate Pheno system drastically reduced the turn around time for the ID and susceptibility testing of positive blood cultures. The time to antimicrobial susceptibility data was reduced from 50.3 hours to 9.1 hours

RESULTS

Figure 1.

Accession Number	Accelerate ID	Blood Culture ID Panel	VITEK MS	Source
10100287-002	CoNS	*Staphylococcus*	*S. epidermidis*	Remnant
10100287-003	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae*	Remnant
10100287-004	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae*	Remnant
10100287-005	*Citrobacter* spp.	N/P	*Citrobacter freundii*	Challenge
10100287-006	*Proteus* spp.	N/P	*Proteus mirabilis*	Challenge
10100287-007	*Pseudomonas aeruginosa*	N/P	*P. aeruginosa*	Challenge
10100287-008	*Enterococcus faecium*	N/P	*E. faecium*	Challenge
10100287-009	*Enterococcus faecalis*	N/P	*E. faecalis*	Challenge
10100287-010	*Klebsiella* spp.	N/P	*Klebsiella oxytoca*	Challenge
10100287-011	*P. aeruginosa*	N/P	*P. aeruginosa*	Challenge
10100287-012	*Acinetobacter baumannii*	N/P	*A. baumannii*	Challenge
10100287-013	*E. faecium*	N/P	*E. faecium*	Challenge
10100287-014	*Staphylococcus aureus*	N/P	*S. aureus*	Challenge
10100287-015	*Escherichia coli*	N/P	*E. coli*	Challenge
10100287-016	*Streptococcus* sp.	Streptococcus, Staphylococcus	*S. parasanguinis, S. epidermidis*	Remnant
10100287-017	*Enterobacter* spp.	N/P	*Enterobacter aerogenes*	Challenge
10100287-018	*S. aureus*	S. aureus	*S. aureus*	Challenge
10100287-019	CoNS	Staphylococcus	*Staphylococcus epidermidis*	Remnant
10100287-020	*S. aureus*	Staphylococcus aureus	*S. aureus*	Remnant
10100287-021	*Citrobacter* spp.	Enterobacteriaceae	*C. freundii*	Remnant
10100287-023	*E. faecium*	N/P	*E. faecium*	Seeded
10100287-024	*E. faecalis*	N/P	*E. faecalis*	Seeded
10100287-025	CoNS	N/P	*Staphylococcus lugdunensis*	Seeded
10100287-026	*S. aureus*	S. aureus	*S. aureus*	Remnant
10100287-027	*E. coli*	E. coli	*E. coli*	Remnant
10100287-028	*P. aeruginosa*	P. aeruginosa	*P. aeruginosa*	Seeded
10100287-029	*A. baumannii*	N/P	*A. baumannii*	Seeded
10100287-030	*Enterobacter* spp.	N/P	*C. freundii*	Seeded
10100287-031	N/D	Escherichia coli	*E. coli*	Seeded
10100287-032	*Serratia marcescens*	N/P	*Serratia marcescens*	Seeded
10100287-033	*Klebsiella* spp.	K. oxytoca	*K. oxytoca*	Seeded
10100287-034	*S. aureus*	S. aureus	*S. aureus*	Seeded
10100287-035	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	Seeded
10100287-036	*E. coli*	E. coli	*E. coli*	Remnant
10100287-037	*Serratia marcescens*	*S. marcescens*	*S. marcescens*	Remnant
10100287-038	N/D	*E. coli*	*E. coli*	Seeded
10100287-039	*Streptococcus* spp.	N/P	*Streptococcus pneumoniae*	Seeded
10100287-040	*Candida albicans*	*C. albicans*	*C. albicans*	Seeded
10100287-041	*Candida glabrata*	*C. glabrata*	*C. glabrata*	Seeded
10100287-042	*Klebsiella* spp.	*K. pneumoniae*	*K. pneumoniae*	Seeded
10100287-043	*Proteus* spp.	*Proteus* spp.	*Proteus mirabilis*	Seeded
10100287-044	CoNS	*Staphylococcus*	*Staphylococcus capitis*	Remnant
10100287-045	*E. coli*	N/P	*E. coli*	Seeded
10100287-046	*Citrobacter* spp.	N/P	*Citrobacter freundii*	Seeded
10100287-047	*E. coli*	N/P	*E. coli*	Seeded
10100287-048	*Proteus* spp.	N/P	*Proteus mirabilis*	Seeded
10100287-049	N/D	N/D	*Stenotrophomonas maltophilia*	Remnant
10100287-050	IND	*Staphylococcus*	*S. epidermidis, P. acnes*	Remnant
10100287-051	*E. coli*	*E. coli*	*E. coli*	Remnant
10100287-052	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	Remnant
10100287-053	N/D	N/D	*Fusobacterium nucleatum*	Remnant
10100287-054	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	Remnant

CoNS – Coagulase Negative Staphylococci
N/P – Not Performed
N/D – Not Detected
IND – Indeterminate

Figure 1. Results for organism identification from the Accelerate Pheno validation study performed at MAMC. The percent positive agreement and percent negative agreement were 91.8% and 99.7% respectively. Six discordant results were obtained, four from seeded specimens and two remnant patient specimen. In two instances the Accelerate system failed to identify *E. coli* in seeded specimens, one specimen was incorrectly identified as an *Enterobacter* sp., and in another the Accelerate identified coagulase negative staphylococcus (CoNS) and, but did not identify it as *S. lugdunensis*. One remnant patient specimen was polymicrobial, the Accelerate did identify *Streptococcus* but failed to detect the CoNS. The second remnant specimens was also polymicrobial with *S. epidermidis* and *P. acnes*, the Accelerate returned an indeterminate result due to low cell numbers.

Figure 2.

Specimen ID	Gram Stain	Accelerate ID	BCID	VITEK MS
1	Gram Negative Rods	*Serratia marcescens*	*S. marcescens, Pseudomonas aeruginosa*	*S. marcescens, P. aeruginosa*
2	Gram Negative Rods	*Escherichia coli*	*E. coli*	*E. coli*
4	Gram Positive Cocci, Clusters	*Staphylococcus. aureus*	*S. aureus*	*S. aureus*
5	Gram Positive Cocci, Chains	CoNS	*Streptococcus*	*Streptococcus*
6	Gram Negative Rods	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
7	Gram Negative Rods	CoNS, *Klebsiella*	*Klebsiella pneumoniae*	*K. pneumoniae*
8	Gram Negative Rods	*Enterobacter*	*Enterobacter cloacae*	*E. cloacae*
9	Gram Negative Rods	*Klebsiella*	*K. pneumoniae*	*K. pneumoniae*
10	Gram Negative Rods, Yeast	*Candida albicans*	*C. albicans,Klebsiella oxytoca*	*C. albicans*
11	Gram Negative Rods	*Klebsiella*	*C. albicans, K. oxytoca*	*C. albicans, K. oxytoca*
12	Gram Positive Cocci, Clusters	*S. aureus*	*S. aureus*	*S. aureus*
13	Gram Negative Rods	*E. coli*	*E. coli*	*E. coli*
14	Gram Negative Rods	*E. coli*	*E. coli*	*E. coli*
15	Gram Negative Rods	N/D	N/D	*Morganella morganii*
16	Gram Positive Cocci, Clusters	*S. aureus*	*S. aureus*	*S. aureus*
17	Gram Negative Rods	N/D	*Haemophilus influenzae*	*H. influenzae*
18	Gram Negative Rods	*Klebsiella*	*K. pneumoniae*	*K. pneumoniae*
19	Gram Positive Cocci	*S. aureus*	*Staphylococcus, S. aureus*	*S. aureus, S. epidermidis*
20	Gram Negative Rods	*Enterobacter, Klebsiella*	*K. pneumoniae*	*K. pneumoniae*
21	Gram Positive Cocci, Clusters	N/D	*Staphylococcus*	*S. epidermidis*
22	Gram Negative Rods	*E. coli*	*E. coli*	*E. coli*
23	Gram Positive Cocci	CoNS	*Staphylococcus*	*S. epidermidis*
24	Gram Positive Cocci, Clusters	CoNS	*Staphylococcus*	*S. epidermidis*
25	Gram Negative Rods	*Klebsiella*	*K. oxytoca*	*K. oxytoca*
26	Gram Negative Rods	*E. coli*	*E. coli*	*E. coli*
27	Gram Positive Cocci, Chains	*Streptococcus*	*S. pyogenes*	*S. pyogenes*
28	Gram Positive Cocci, Clusters	CoNS	*Staphylococcus*	*Staphylococcus warneri*
29	Gram Negative Rods	CoNS	*P. aeruginosa*	*P. aeruginosa*
30	Gram Negative Rods	*E. coli*	*E. coli*	*E. coli*
31	Gram Positive Cocci, Clusters	*S. aureus*	*S. aureus*	*S. aureus*

CoNS – Coagulase Negative Staphylococci
N/D – Not Detected

Figure 2. Results for part two of the study which was a retrospective data collection with the Accelerate Pheno being run in conjunction with the MAMC laboratories standard method. Thirty specimens were analyzed over an approximate 10 week period. The percent positive agreement and percent negative agreement were 80.6% and 99.0% respectively. Review of the instrument data by the manufacturer suggest that false negative results were the result of either low cells numbers or instrument issues. The false positive results may have been the result of debris. Polymicrobial failures likely are the result of multiple contributing factors (e.g. low cell numbers of the target organism and debris).

Figure 3.

A.

Essential Agreement (N)	Essential Agreement	Categorical Agreement (N)	Categorical Agreement	VME	ME	MiE
224	215 (96.0%)	229	215 (93.9%)	2	0	12
Error Classification	**Organism**	**Antibiotic**	**Pheno MIC (µg/ml)**	**Vitek2 MIC (µg/ml)**	**BMD MIC (µg/ml)**	
VME 1	*P. aeruginosa*	Cefepime	4 (S)	≥32 (R)	16 (I)	
VME 2	*Citrobacter* sp	Piperacillin-Tazobactam	8 (S)	128 (R)	8 (S)	

B.

Essential Agreement (N)	Essential Agreement	Categorical Agreement (N)	Categorical Agreement	VME	ME	MiE
104	96 (92.3%)	108	102 (98.1%)	0	2	4
Error Classification	**Organism**	**Antibiotic**	**Pheno MIC (µg/ml)**	**Vitek2 MIC (µg/ml)**	**BMD MIC (µg/ml)**	
ME 1	*K. oxytoca*	Ampicillin-Sulbactam	32 (R)	8 (S)	NP	
ME 2	*K. oxytoca*	Ampicillin-Sulbactam	32 (R)	8 (S)	NP	

VME – Very Major Error, ME – Major Error, MiE – Minor Error, NP-Not Performed

Figure 3. Summary of the antimicrobial susceptibility testing results (AST) for the validation (A.) and prospective studies (B.). During the validation study 2 very major errors (VME) and 12 minor errors (MiE) were identified. The VMEs were adjudicated by broth micro dilution (BMD). Following adjudication VME 1 would have been a minor error, and the Pheno result was in agreement with the BMD result for VME 2. VMEs in the prospective study were not further tested. Two VMEs and 4 MiEs were identified. Both VMEs occurred in *K. oxytoca* isolates when testing Ampicillin-Sulbactam resistance.

Conclusions

- The Accelerate Pheno system has been implemented in the MAMC Microbiology laboratory
- There has been a dramatic decrease in the turn-around-time for antimicrobial susceptibility results
- Some identification issues
  - Low cell numbers
  - Debris
  - System not fully integrated
- Continuing to use the system and monitor performance