Analysis of the FAST-Prep™ PBC System for expedited identification and susceptibilities of bloodborne pathogens

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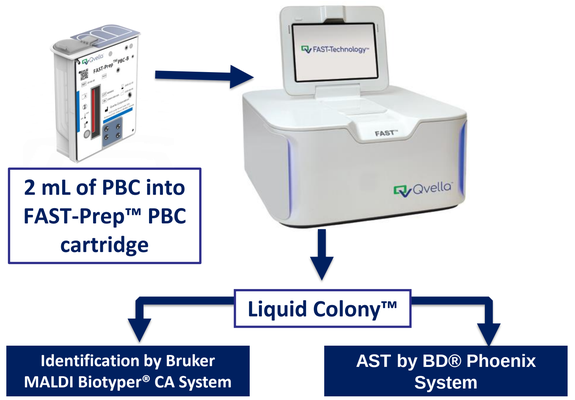
INTRODUCTION

Due to the high morbidity and mortality associated with sepsis there is interest in developing rapid diagnostics to improve turnaround time to results for both identification (ID) and antimicrobial susceptibility testing (AST) in positive blood cultures (PBC). Conventional subculture techniques can take at a minimum 24-48 hours on average before isolates are available for use for further testing. In this study we evaluated the Qvella FAST-Prep™ PBC System (Qvella, Ontario, Canada) compared to standard of care subculture for ID and AST. FAST-Prep™ PBC is an automated system that isolates and concentrates pathogenic cells from a positive blood culture. Total run time is ~30 minutes for 1 cartridge and ~40 minutes for 2 cartridges. The resulting Liquid Colony™ (LC) can be used directly with downstream ID and AST applications within the microbiology laboratory.

OBJECTIVES

Beta analysis of FAST-Prep™ PBC System included 181 positive blood cultures, 153 prospective samples and 28 contrived specimens, collected at the University of California, San Diego. Upon positivity the blood culture was processed per standard of care (SOC) and using FAST-Prep. Two ml of PBC was added to the FAST-Prep cartridge. Resulting LC was tested for ID using the Biotyper MALDI-Tof MS (Bruker Daltonics, Billerica, MA) and AST using the Phoenix (Becton Dickinson, Franklin Lakes, New Jersey) along with an isolated colony on agar media per SOC.

FAST-Prep™ PBC System WORKFLOW



Gram stain and subculture performed on all PBC

METHOD

Positive Blood Cultures

Prospective PBCs and Contrived specimens

- Incubated in BD BACTEC™ FX Instruments
- Prospective samples: collected from October 2020-March 2021
- Contrived specimens: prepared by adding 500µ l of either a 0.25 or 0.5 McF preparation of each isolate to previously verified negative blood culture
- Polymicrobial cultures identified on Gram stain were excluded from analysis

FAST-Prep™ PBC System Workflow

Positive Blood Culture → 30-40 min → Liquid Colony™ → 1µl LC Maldi / 0.25 – 0.5 McF Suspension → BD Phoenix® AST

Standard of Care Workflow

Positive Blood Culture → overnight → Overnight sub culture → Maldi / 0.25 – 0.5 McF Suspension → BD Phoenix® AST

Enterobacterales Antibiotics	
Amikacin	Gentamicin
Ampicillin	Meropenem
Ampicillin/Sulbactam	Piperacillin/Tazobactam
Cefazolin	Tobramycin
Cefoxitin	Trimethoprim Sulfamethoxazole
Ceftazidime	
Ceftriaxone	
Ciprofloxacin	
Ertapenem	

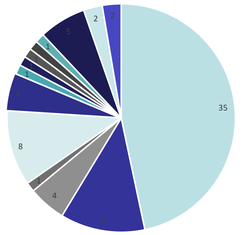
Pseudomonas Antibiotics
Amikacin
Aztreonam
Cefepime
Ceftazidime
Ciprofloxacin
Gentamicin
Meropenem
Piperacillin/Tazobactam
Tobramycin

Staphylococcus Antibiotics	*Streptococcus* Antibiotics
Cefazolin	Ceftriaxone
Clindamycin	Clindamycin
Daptomycin	Penicillin
Erythromycin	
Linezolid	
Oxacillin	
Rifampin	
Tetracycline	
Vancomycin	

Enterococcus Antibiotics
Ampicillin
Linezolid
Piperacillin
Tetracycline
Vancomycin

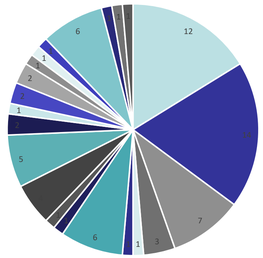
RESULTS

Gram Negative: Prospective with Seeded Organisms



- Escherichia coli
- Klebsiella pneumoniae
- Pseudomonas aeruginosa
- Citrobacter koseri
- Serratia marcescens
- Enterobacter cloacae
- Psychrobacter sanguinis
- Raoultella ornithinolytica
- Acinetobacter courvalinii
- Klebsiella variicola
- Achromobacter xylosoxidans
- Proteus mirabilis
- Enterobacter spp.
- Klebsiella aerogenes

Gram Positives: Prospective with Seeded Organisms



- Staphylococcus aureus
- Staphylococcus epidermidis
- Streptococcus pyogenes
- Staphylococcus haemolyticus
- Peptoniphilus harei
- Micrococcus luteus
- Enterococcus faecalis
- Bacillus cereus
- Streptococcus gordonii
- Staphylococcus hominis
- Enterococcus faecium
- Streptococcus mitis
- Enterococcus gallinarum
- Staphylococcus capitis
- Streptococcus gallolyticus
- Nosocomiicoccus massiliensis
- Streptococcus anginosus
- Staphylococcus pettenkoferi
- Streptococcus agalactiae
- Streptococcus oralis
- Rothia dentocariosa
- Streptococcus constellatus

Prospective and Seeded Combined — BD Phoenix® AST

		Total	Total test #	EA	CA	S	I	R	minE	MajE	VME
GP Bacteria	n=	62	391	389	390	334	3	54	1	0	0
	%			99.5%	99.7%						
GN Bacteria	n=	72	1027	1008	1005	857	20	150	18	2	2
	%			98.1%	97.9%						

Prospective Only — BD Phoenix® AST

		Total	Total test #	EA	CA	S	I	R	minE	MajE	VME
GP Bacteria	n=	49	342	340	341	287	3	52	1	0	0
	%			99.4%	99.7%						
GN Bacteria	n=	57	832	819	814	696	19	117	15	1	2
	%			98.4%	97.8%						

Seeded Only — BD Phoenix® AST

		Total	Total test #	EA	CA	S	I	R	minE	MajE	VME
GP Bacteria	n=	13	49	49	49	47	0	2	0	0	0
	%			100%	100%						
GN Bacteria	n=	15	195	189	191	161	1	33	3	1	0
	%			96.9%	97.9%						

CA; categorical agreement, EA; essential agreement, mE; minor error, ME; major error; VME; very major error

- Of the 181 samples tested 12 runs were invalid, 9 were removed because they were a mixed culture and 1 was a false positive (high WBC).
- Of the remaining 159 samples, 8 could not be identified using the LC, 5 resulted in an ID but no AST, 3 samples were inconclusive by SOC Maldi-Tof MS.
- Samples with no LC ID (n=8) included *Serratia marcescens, Corynebacterium imitans, Clostridium ramosum, Corynebacterium simulans, Actinomyces turicensis, Streptococcus oralis, Corynebacterium sp.* and *Enterococcus casseliflavus*
- Samples generating ID but no AST using LC (n=5) included *3 Staphylococcus epidermidis, Acinetobacter sp. and Streptococcus pyogenes*
- 148/148 (100%) of specimens that generated an ID using Liquid Colony and a valid result by SOC MALDI-Tof MS were concordant.
- Two Gram-negative VME were seen with 1 *E. coli* and 1 *Enterobacter sp.* with cefazolin.

CONCLUSIONS

- Of the 148 samples where ID was obtained correlation was equivalent (100% agreement) to SOC.
- CA and EA for AST were very high compared to SOC testing. AST results for combined Gram-positive bacteria showed a CA of 99.7% and an EA of 99.5%. AST results for combined Gram-negative bacteria showed a CA of 97.9% and an EA of 98.1%.
- Though 2 VME were seen with *E. coli* and *Enterobacter sp.*, cefazolin is not an antibiotic routinely used in our institution for the treatment of Gram-negative bacteremia.
- The FAST-Prep™ PBC can accelerate ID and phenotypic AST results directly from positive blood cultures by approximately 24-48 hours compared to the SOC and can potentially impact the time to effective therapy in patients with sepsis.

CLSI. 2020. Performance standards for antimicrobial susceptibility testing. 29th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.