Evaluation of a Novel Sample Preparation Method for Rapid Antimicrobial Resistance Testing Directly from Positive Blood Cultures

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ABSTRACT

Qvella (Richmond Hill, Canada) has developed the FAST™ System, an automated centrifugal sample preparation system that rapidly produces a Liquid Colony™ (LC) consisting of a purified, concentrated, viable cell suspension directly from a positive blood culture (PBC). The resulting LC may potentially be used for identification (ID) and antimicrobial susceptibility testing (AST), producing results one day earlier compared to the standard of care (SOC) diagnostic workflow requiring subculturing a PBC on solid media. This study was performed to evaluate the feasibility of using the LC for selected rapid antimicrobial resistance tests: Clearview™ PBP2a SA Culture Colony Test (Abbott, Abbott Park, IL), β LACTA™ test and β CARBA™ test (Bio-Rad, Marnes-la-Coquette, France).

OBJECTIVES

Rapid detection of resistance in microorganisms can aid in the clinical management of patients with serious infections. Several methodologies exist for the rapid detection of enzyme mediating antimicrobial resistance from bacterial culture growth prior to the phenotypic AST being available. Examples of these rapid resistance tests are the PBP2a test for the detection of MRSA, β LACTA™ test which detects bacteria with reduced susceptibilty to third generation cephalosporins, and the β CARBA™ test which detects bacterial strains with reduced susceptibility to carbapenems due to the production of carbapenemases. The objective of this study was to determine the feasibility of performing these tests using the LC produced by the FAST-PBC Prep™ System for the rapid detection of resistance from positive blood cultures.

FAST-PBC Prep™ System WORKFLOW

2 ml of PBC into FAST-PBC Prep™ cartridge

Liquid Colony™

Identification by Vitek® MS System

AST by Vitek® 2 System

METHOD

Contrived PBC samples were prepared by spiking species of Gram-positive and Gram-negative bacteria into BACT/ALERT® Plus bottles and incubating in the BACT/ALERT® VIRTUO® System (bioMérieux, Durham, NC). After blood culture positivity, 2 ml of PBC was processed using the FAST™ System and FAST-PBC Prep™ Cartridge. After ~21 minutes of processing, the LC was tested for ID and AST using VITEK®MS and VITEK®2 (bioMérieux, Durham, NC), respectively. For PBP2a testing, ~50 µL of LC was inoculated onto a blood agar plate (BAP) and incubated at 37ºC for 2 hours prior to testing. For the β LACTA™ test and β CARBA™ test, ~50 µL of LC was centrifuged at 13,000g for 1 min and the pellet was used to perform each test. SOC subculture colonies of respective isolates were tested per the manufacturer package insert.

Liquid Colony™

PBP2a Testing
50 µl of LC inoculated onto a BAP

Incubate at 37°C for 2 hours

Perform PBP2a on solid colony from BAP

β LACTA™ & β CARBA™ testing 50 µl of LC centrifuged for 13,000 rpm for 1 min

Pellet used to perform β LACTA™ and β CARBA™ tests

*SOC colonies were tested per manufacturer package inserts

RESULTS

Of the 11 MRSA and 9 MSSA strains, 91% (10/11) were PBP2a positive and 100% (9/9) PBP2a™ negative for SOC and LC. When comparing SOC and LC (2hr BAP plated colony) there was 100% concordance using the PBP2a™ test.

Of the 13 β-lactamase positive and 6 β-lactamase negative Enterobacteriaceae, 92% (12/13) were β LACTA™ positive and 100% (6/6) β LACTA™ negative for LC and SOC. One *E.* cloacae was R to ceftriaxone that was not detected by the β LACTA™ test using the SOC or the LC. When comparing SOC and LC there was 100% concordance using the β LACTA™ test.

Of the 6 carbapenemase negative and 3 carbapenemase positive Enterobacteriaceae strains there was 100% concordance between the SOC and LC using the β CARBA™ test.

RESULTS

Table 1. PBP2a SOC vs Liquid Colony™ (2 hr Growth)

		PBP2A-Abbott Testing		VITEK2 AST (Reference)		
S. aureus Strain	MSSA/MRSA	SOC	LC-2 hours on agar	Cefoxitin Screen	Oxacillin	
				MIC	MIC	Interp.
1	MSSA	NEG	NEG	NEG	<0.25	S
2	MSSA	NEG	NEG	NEG	<0.25	S
3	MSSA	NEG	NEG	NEG	<0.25	S
4	MSSA	NEG	NEG	NEG	<0.25	S
5	MSSA	NEG	NEG	NEG	0.5	S
6	MSSA	NEG	NEG	NEG	<0.25	S
7	MSSA	NEG	NEG	NEG	0.5	S
8	MSSA	NEG	NEG	NEG	<0.25	S
9	MSSA	NEG	NEG	NEG	0.5	S
10	MRSA	POS	POS	POS	>4	R
11	MRSA	POS	POS	POS	>4	R
12	MRSA	NEG	NEG	POS	1	S
13	MRSA	POS	POS	POS	>4	R
14	MRSA	POS	POS	POS	>4	R
15	MRSA	POS	POS	POS	>4	R
16	MRSA	POS	POS	POS	>4	R
17	MRSA	POS	POS	POS	>4	R
18	MRSA	POS	POS	POS	>4	R
19	MRSA	POS	POS	POS	>4	R
20	MRSA	POS	POS	POS	>4	R

MSSA = Methicillin Sensitive *Staphylococcus aureus*
MRSA = Methicillin Resistant *Staphylococcus aureus*

Table 2. β LACTA™ test SOC vs Liquid Colony™ Pellet

Test Microorganism		β LACTA Test		VITEK®2 AST (Reference)							
Species	Strain	SOC	LC-pellet	Cefazolin		Cefoxitin		Ceftazidime		Ceftriaxone	
				MIC	Interp.	MIC	Interp.	MIC	Interp.	MIC	Interp.
Escherichia coli	1	NEG	NEG	≤4	S	≤4	S	≤0.12	S	≤0.25	S
	2	POS	POS	≥64	R	≥4	R	≥64	R	≥64	R
	3	POS	POS	≥64	R	≥4	R	32	R	≥64	R
	4	POS	POS	≥64	R	≥4	R	≥64	R	≥64	R
	5	POS	POS	≥64	R	≥4	R	8	I	≥64	R
Klebsiella pneumoniae	6	NEG	NEG	≤4	S	≤4	S	0.25	S	≤0.25	S
	7	POS	POS	≥64	R	32	R	≥64	R	4	R
	8	POS	POS	≥64	R	≥64	R	32	R	≥64	R
	9	POS	POS	≥64	R	≥4	R	32	R	≥64	R
	10	POS	POS	≥64	R	≥4	R	32	R	≥64	R
Acinetobacter baumannii	11	NEG	NEG	≥64	R			16	I	16	I
	12	NEG	NEG	≥64	R			4	S	4	S
	13	POS	POS	≥64	R			≥64	R	≥64	R
Enterobacter cloacae	14	NEG	NEG	≥64	R	≥64	R	4	S	32	R
	15	NEG	NEG	≥64	R	≥64	R	0.25	S	≤0.25	S
	16	POS	POS	≥64	R	≥64	R	≥64	R	≥64	R
	17	POS	POS	≥64	R	≥4	R	≥64	R	≥64	R
Proteus mirabilis	18	NEG	NEG	≥64	R	16	I	≤1	S	≤1	S
	19	POS	POS	≥64	R	≤4	S	≥64	R	≥64	R

Table 3. β CARBA™ test SOC vs Liquid Colony™ Pellet

Test Microorganism		β CARBA Test		VITEK®2 AST					
Species	Strains	SOC	LC-pellet	Ertapenem		Imipenem		Meropenem	
				MIC	Interp.	MIC	Interp.	MIC	Interp.
Klebsiella pneumoniae	1	NEG	NEG	≤0.12	S	0.5	S	≤0.25	S
	2	NEG	NEG	≤0.12	S	0.5	S	≤0.25	S
	3	POS	POS	≥8	R	≥16	R	≥16	R
Acinetobacter baumannii	4	NEG	NEG			≤0.25	S	2	S
	5	NEG	NEG			≤0.25	S	≤0.25	S
	6	POS	POS			≥16	R	≥16	R
Enterobacter cloacae	7	NEG	NEG	≤0.12	S	1	S	≤0.25	S
	8	NEG	NEG	≤0.25	S	≤0.25	S	≤0.25	S
	9	POS	POS	≥8	R	≥16	R	≥16	R

CONCLUSIONS

- All but 1 of the MRSA strains were PBP2a positive using both the overnight SOC colony and the LC incubated at 2 hours on BAP. One MRSA (based on cefoxitin screen) was PBP2a negative by both the SOC overnight colony and LC. This isolate did have a reduced MIC to oxacillin of 1 µg/ml.
- All but 1 (*E. cloacae*) of the β –lactamase positive strains by Vitek 2 were β LACTA™ positive by both SOC and LC. Testing using the β CARBA™ test showed 100% concordance between SOC and LC. Number of samples assayed using the β CARBA™ test is small therefore further testing is warranted.
- These studies show the feasibility of using the LC in rapid resistance testing methods available on the market today.
- Any downstream use or deviation from the manufacturers' package insert would need to be validated in each individual laboratory setting.

[1]CLSI. 2021. Performance standards for antimicrobial susceptibility testing. 29th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA