Comparison of a Novel Sample Preparation Method for Positive Blood Cultures vs. Standard of Care Overnight Sub-Culture: 0.5 McFarland Cell Concentrations and Antimicrobial Susceptibility Test Results

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ABSTRACT

Phenotypic antimicrobial susceptibility testing (AST) of bacteria from positive blood cultures (PBC) involves preparation of a 0.5 McFarland inoculum from overnight subcultured colonies. Qvella (Richmond Hill, ON, CA) has developed the FAST™ System, an automated centrifugal sample preparation system which delivers a Liquid Colony™ (LC) consisting of a purified, concentrated, viable cell suspension directly from a PBC in ~21 minutes. The FAST™ System-derived LC allows for an AST to be run 1 day earlier, potentially enabling earlier reporting of phenotypic AST results. This study compared cell concentrations in the 0.5 McFarland suspension prepared using both the LC and the standard of care (SOC) subculture. AST results obtained using the LC and SOC inoculum were also compared.

OBJECTIVES

The objective of this study was to compare cell concentrations in a 0.5 McFarland (McF) generated using the LC from the FAST-PBC Prep™ System to the cell concentrations in a 0.5 McF generated using a SOC overnight colony. In addition, AST results from the Vitek® 2, using both the LC and SOC generated McF, were compared using the Vitek® 2.

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

In this study contrived PBCs were prepared by spiking 8 species of Gram-positive and 10 species of Gram-negative bacteria BACT/ALERT® Plus bottles and incubating in the BACT/ALERT® VIRTUO® System (bioMérieux, Durham, NC) and BACTEC™ Plus bottles incubating them in the BACTEC™ FX System (Becton Dickinson, Franklin Lakes, NJ). Approximately 2hr after positivity, 2 ml of PBC for each bottle was processed in the FAST System. Serial dilutions of the 0.5 McFarland suspensions prepared using the LC and SOC colony were plated and incubated overnight to determine colony counts. LC and SOC overnight subculture colonies were used to prepare 0.5 McFarland inocula for the VITEK®2 AST. Essential and categorial agreement (EA and CA respectively) were calculated comparing the LC to SOC AST.

Colony Counts of both 0.5 McF LC and SOC overnight subculture isolate

Prepare 0.5 McF using LC and SOC overnight culture colony

↓

Perform 2 fold serial dilutions from LC and SOC 0.5 McF

↓

Plate to BAP

↓

Perform Colony Counts

RESULTS

Colony counts obtained with serial dilutions of the LC and SOC 0.5 McF are shown in Tables 1 & 2. Colony counts comparing LC to SOC overnight colony were very similar for samples seeded into both bioMerieux and BD bottles. Vitek 2 AST was peformed using the 17 organisms in Tables 1 & 2 and was performed using the LC obtained from seeded bioMerieux blood culture bottles summarized in Table 3. AST EA and CA using the LC compared to SOC was 97.2% and 95.2%, respectively, for Gram-positives (GP67 card); 100% and 100%, respectively, for Streptococci (ST03 card) and 97.9% and 97.5%, respectively, for Gram-negatives (N392 card).

RESULTS

Table 1. Colony Counts using bioMérieux Culture Bottles

Species	Bottle Type bioMérieux	LC 0.5 MF CFU/mL	SOC 0.5 MF CFU/mL	LC/SOC Ratio
Enterococcus faecalis	FA	1.0E+08	8.0E+07	1.3
Enterococcus faecium	FA	7.9E+07	1.0E+08	0.8
Staphylococcus aureus	FA	2.0E+06	6.7E+07	0.03
Staphylococcus epidermidis	FA	3.5E+06	5.6E+07	0.06
Streptococcus agalactiae	FA	1.8E+08	8.8E+07	2.0
Streptococcus pneumoniae	FA	1.3E+08	1.0E+07	13.4
Streptococcus mitis/oralis	FA	1.8E+07	4.3E+07	0.4
Streptococcus sanguinis	FA	1.1E+07	4.4E+07	0.2
		6.6E+07	**6.1E+07**	**1.1**
Acinetobacter baumannii	FA	8.6E+07	9.4E+07	0.9
Enterobacter cloacae Complex	FA	1.9E+08	1.3E+08	1.4
Escherichia coli	FA	1.3E+08	9.4E+07	1.4
Klebsiella pneumoniae	FA	1.1E+08	4.3E+07	2.6
Pseudomonas aeruginosa	FA	5.3E+07	1.1E+08	0.5
Serratia marcescens	FA	9.5E+07	2.3E+08	0.4
Proteus mirabilis	FA	1.2E+08	1.5E+08	0.8
Citrobacter freundii	FA	1.3E+08	1.3E+08	1.0
Stenotrophomonas maltophilia	FA	5.0E+07	1.2E+08	0.4
Bacteroides fragilis	FN	4.0E+07	1.2E+08	0.3
		1.0E+08	**1.2E+08**	**0.8**

Table 2. Colony Counts using BD Culture Bottles

Species	Bottle Type BD	LC 0.5 MF CFU/mL	SOC 0.5 MF CFU/mL	LC/SOC Ratio
Enterococcus faecalis	AP	1.2E+08	5.1E+07	2.3
Enterococcus faecium	AP	1.1E+08	6.0E+07	1.9
Staphylococcus aureus	AP	7.5E+05	7.1E+07	0.01
Staphylococcus epidermidis	AP	7.8E+06	2.3E+07	0.3
Streptococcus agalactiae	AP	1.8E+08	4.4E+07	4.2
Streptococcus pneumoniae	AP	9.6E+07	2.1E+07	4.6
Streptococcus mitis/oralis	AP	2.3E+07	5.7E+07	0.4
Streptococcus sanguinis	AP	1.4E+07	5.3E+07	0.3
		6.9E+07	**4.7E+07**	**1.5**
Acinetobacter baumannii	AP	9.1E+07	7.2E+07	1.3
Enterobacter cloacae Complex	AP	1.1E+08	1.9E+08	0.6
Escherichia coli	AP	8.5E+07	1.7E+08	0.5
Klebsiella pneumoniae	AP	1.5E+08	8.6E+07	1.7
Pseudomonas aeruginosa	AP	5.4E+07	1.2E+08	0.4
Serratia marcescens	AP	4.5E+07	2.7E+07	1.7
Proteus mirabilis	AP	1.2E+08	1.7E+08	0.7
Citrobacter freundii	AP	1.2E+08	7.3E+07	1.6
Stenotrophomonas maltophilia	AP	6.1E+07	1.4E+08	0.4
Bacteroides fragilis	AN	1.9E+07	1.2E+08	0.2
		8.5E+07	**1.2E+08**	**0.7**

Table 3. AST Results using Vitek® 2 and Liquid Colony (bioMérieux Culture Bottle)

Gram +/-	Bug/Drug Combinations	CA	EA	S	I	R	minE	MajE	VME
Gram + (n=4)	124	118 (95.2%)	105 (97.2%)	105	10	9	5	0	1*
Strep (n=4)	120	120 (100%)	118 (100%)	116	2	2	0	0	0
Gram – (n=8)***	236	230 (97.5%	231(97.9%)	201	2	33	5	1**	0

*Staphylococcus aureus/Benzylpenicillin
**_Proteus mirabilis_/Imipenem
***No AST data was obtained for _B. fragilis_

CONCLUSIONS

- Cell concentrations measured in 0.5 McFarland suspensions prepared from the LC and the SOC sub-cultured colony were similar.
- Gram-positive cocci such as *S. aureus/S. epidermidis* are sometimes clumped on appearance when the LC is Gram stained. This could account for a slightly lower colony count versus SOC for these organisms.
- AST results obtained with the VITEK®2 automated AST system using a 0.5 McFarland inoculum derived from the LC were comparable to those obtained with the SOC method with 95% or greater EA and CA.
- FAST-PBC Prep™ System generated LC is a potential solution to accelerate workflow and obtain an ID the same day using MALDI-Tof and a phenotypic AST result 24 hours or earlier.
- 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