

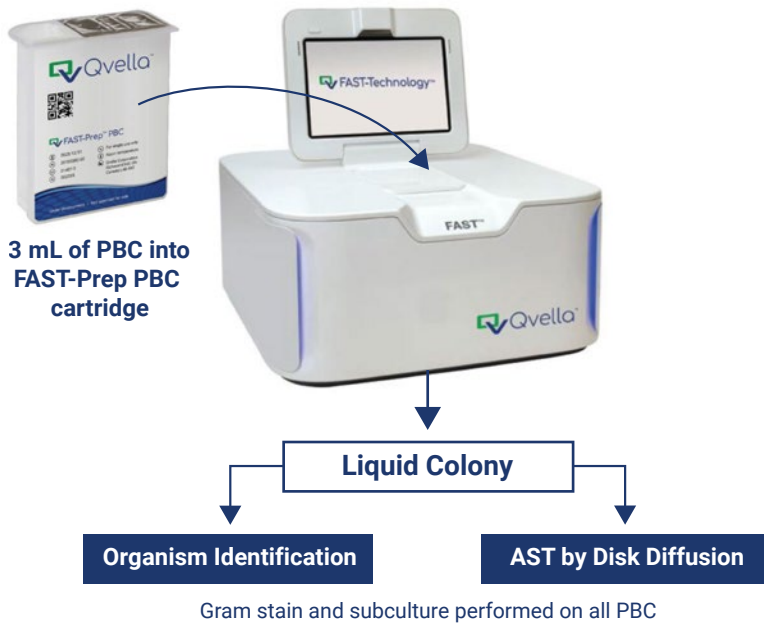
BACKGROUND

Conventional antimicrobial susceptibility testing (AST) of microorganisms from positive blood cultures (PBC) can take ≥ 2 days. In order to improve the turnaround time for AST on a PBC, CLSI and EUCAST have made efforts to standardize procedures for disk diffusion (DD) direct from a PBC. Qvella (Richmond Hill, ON, CA) has developed the FAST Prep™ System, an automated centrifugal sample preparation system that rapidly delivers a Liquid Colony: a clean, concentrated, viable pathogen cell suspension directly from a PBC. This study was performed to investigate the feasibility of DD AST using a Liquid Colony.

OBJECTIVES

- Compare the biomass from a Liquid Colony with the biomass in a positive blood culture.
- Compare the biomass in 0.5 McFarland derived from Liquid Colony with a 0.5 McFarland derived from a solid colony.
- Verify that a 0.5 McFarland suspension, from a Liquid Colony can be used as the inoculum for conventional DD testing (using CLSI M 100 interpretative criteria).

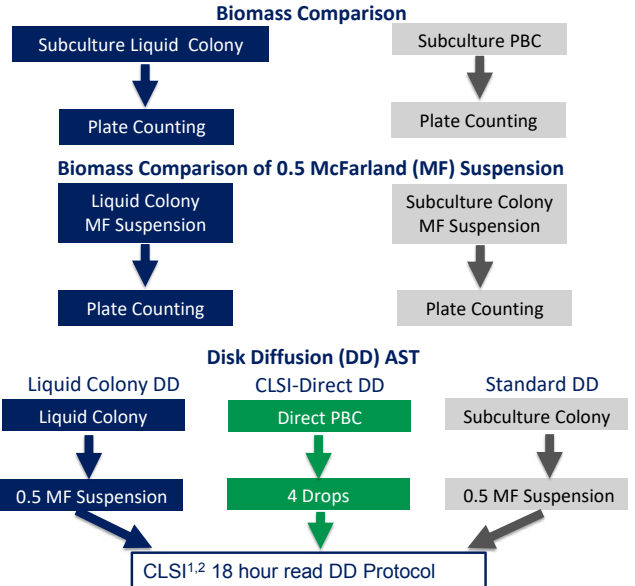
FAST-Prep™ WORKFLOW



METHOD

Contrived PBC Preparation and Processing

- Spike SPS whole blood with <10 CFU/mL bacteria
- Inoculate BacT/ALERT® FA Plus bottles
- Incubate in BacT/ALERT® VIRTUO® System



Gram-Positive Bacteria Susceptibility Panels		
<i>Enterococcus</i> spp.	<i>Staphylococcus</i> spp.	<i>Streptococcus</i> spp.
Ampicillin	Cefoxitin	Clindamycin
Ciprofloxacin	Clindamycin	Erythromycin
Erythromycin	Erythromycin	Levofloxacin
Levofloxacin	Levofloxacin	SXT
Tetracycline	Tetracycline	
Vancomycin	SXT	

Gram-Negative Bacteria Susceptibility Panels		
<i>Enterobacteriaceae</i>	<i>P. aeruginosa</i>	<i>Haemophilus</i> spp.
<i>Acinetobacter</i> spp.	Amikacin	Ampicillin
Amikacin	Ceftazidime	Ciprofloxacin
Ceftazidime	Ciprofloxacin	Levofloxacin
Ciprofloxacin	Levofloxacin	SXT
Meropenem	Meropenem	
Piperacillin/Tazobactam	Piperacillin/Tazobactam	
SXT		

RESULTS

Table 1. Average Biomass in PBC and Liquid Colony

Test Sample	Species	Strain (n)	CFU/mL	
			PBC	Liquid Colony
<i>Enterococcus faecalis</i>		5	2.87×10^9	1.11×10^9
<i>Enterococcus faecium</i>		5	4.84×10^8	2.04×10^9
<i>Staphylococcus aureus</i>		5	2.83×10^7	7.79×10^7
<i>Staphylococcus lugdunensis</i>		4	5.54×10^7	3.07×10^8
<i>Streptococcus pneumoniae</i>		4	4.54×10^8	4.99×10^8
<i>Streptococcus pyogenes</i>		3	9.85×10^8	2.89×10^8
Gram-positive average			8.13×10^8	7.20×10^8
<i>Escherichia coli</i>		3	1.78×10^9	4.03×10^9
<i>Acinetobacter baumannii</i>		3	3.02×10^8	4.82×10^8
<i>Pseudomonas aeruginosa</i>		4	4.52×10^8	3.72×10^8
<i>Haemophilus influenzae</i>		3	1.87×10^9	5.64×10^8
Gram-negative average			1.04×10^9	1.23×10^9

Table 2. Average Concentrations in Standardized 0.5 MF Suspensions

Test Sample	Species	Strain (n)	CFU/mL	
			Subculture	Liquid Colony
<i>Enterococcus faecalis</i>		5	4.78×10^7	6.82×10^7
<i>Enterococcus faecium</i>		5	7.66×10^7	5.38×10^7
<i>Staphylococcus aureus</i>		5	7.44×10^7	3.08×10^6
<i>Staphylococcus lugdunensis</i>		4	9.80×10^7	1.42×10^7
<i>Streptococcus pneumoniae</i>		4	5.78×10^7	6.80×10^7
<i>Streptococcus pyogenes</i>		3	5.77×10^7	1.95×10^7
Gram-positive average			6.87×10^7	3.78×10^7
<i>Escherichia coli</i>		3	9.27×10^7	7.60×10^7
<i>Acinetobacter baumannii</i>		3	6.80×10^7	3.43×10^7
<i>Pseudomonas aeruginosa</i>		4	2.50×10^8	5.60×10^7
<i>Haemophilus influenzae</i>		3	9.80×10^7	9.47×10^7
Gram-negative average			1.15×10^8	5.98×10^7

Table 3. Disk Diffusion Antimicrobial Susceptibility Test Comparison

Test Sample	Category	Strain (n)	Test (n)	DD from FAST-Prep Liquid Colony					
				CA	S	I	R	mE	ME
Gram-positive bacteria		25	136	130	80	19	33	6	0
	%			95.6				4.4	0.0
Gram-negative bacteria		13	72	71	49	11	12	1	0
	%			98.6				1.4	0.0

Test Sample	Category	Strain (n)	Test (n)	CLSI-Direct DD					
				CA	S	I	R	mE	ME
Gram-positive bacteria		25	136	105	62	22	48	28	3
	%			77.2				20.6	4.8
Gram-negative bacteria		13	72	59	38	20	13	12	1
	%			81.9				16.7	2.6

CONCLUSIONS

- Biomass obtained from Liquid Colony is sufficient to prepare a 0.5 McFarland suspension for DD susceptibility testing.
- DD AST using a Liquid Colony resulted in a CA of $\geq 95\%$ and improved performance than CLSI Direct DD when compared to standard DD AST resulting in no ME and VME.
- FAST Prep™ PBC generated Liquid Colony can be potentially used to set up an AST 24 hours earlier than a conventional subculture colony.

¹CLSI. 2018. Performance standards for antimicrobial disk susceptibility tests. 13th ed. CLSI supplement M02. Clinical and Laboratory Standards Institute, Wayne, PA.

²CLSI. 2019. Performance standards for antimicrobial susceptibility testing. 29th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.

³Chandrasekaran, S., et. al. 2018. Direct from blood culture disk diffusion to determine antimicrobial susceptibility of gram negative bacteria: preliminary report from the Clinical and Laboratory Standards Institute methods development and standardization working group. J. Clin. Microbiol. 56:1-10.

The FAST-Prep™ PBC System is under development and not approved for sale. The performance characteristics have not been established. VITEK® MS and VITEK® 2 are trademarks of bioMérieux SA or its subsidiaries. BD Vacutainer® SPS is a trademark of Becton Dickinson and Co.