# A Novel Sample Preparation Method for Positive Blood Cultures: Assessment of Sample Stability for Downstream Identification and Antimicrobial Susceptibility Testing

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## ABSTRACT

Standard of care (SOC) workflow for positive blood cultures (PBCs) includes overnight subculturing on solid media for identification (ID) and antimicrobial susceptibility testing (AST). Qvella (Richmond Hill, Canada) has developed the FAST™ System, an automated sample preparation system which generates a Liquid Colony™ (LC). The LC consists of a purified, concentrated, viable cell suspension obtained from the PBC. The LC can potentially be used for ID/AST up to 1 day earlier than SOC subculture. This study examined stability of the LC held at room temperature (RT) for <1hr, 3hr and 5hr for ID/AST.

## OBJECTIVES

Based on different workflows in the clinical laboratory there might be delay in the clinical laboratory in using the LC for ID and or AST. Automated susceptibility systems in addition to methods such as Disk Diffusion and microbroth dilution have guidelines for how long a McFarland preparation can sit at RT before use. This feasibility study was designed to look at LC performance with ID and AST after <1hr, 3hr and 5hr at RT.

## FAST-PBC Prep™ System WORKFLOW

2 ml of PBC into FAST-PBC Prep™ cartridge


**Liquid Colony™**

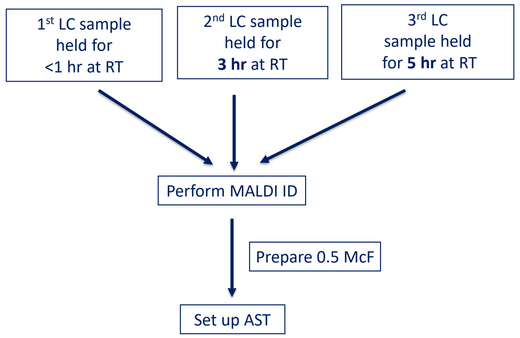
**Identification by Vitek® MS System**

**AST by Vitek®2 System**

## METHOD

Contrived PBCs were prepared by spiking 8 species of Gram-positive and 8 species of Gram-negative bacteria into BACT/ALERT® Plus bottles bottles and incubating in the BACT/ALERT® VIRTUO® (bioMérieux, Durham, NC). After the bottle was flagged positive, 2 ml of PBC were added to the FAST-PBC Prep™ Cartridge and processed with the FAST™ System. After procesing (same blood culture 3 separate times), the LC was removed and held for <1 hr at RT, 3hrs at RT, and 5hr at RT priot to ID and AST. For ID, 0.5µl of LC was spotted to the VITEK® MS template with the addition of matrix and Formic Acid. For AST, the LC was used to prepare a 0.5 McFarland (McF) suspension and assayed using the GP67, ST03 and N392 VITEK®2 cards. Correct ID, Essential Agreement (EA) and Categorial Agreement (CA) were calculated compared to SOC.

**Liquid Colony™ – 3 separate FAST-PBC Prep™ runs/same PBC**




## RESULTS

Of the microorganisms tested (Table 1) the overall ID/AST results for LC held at RT for various time periods are shown in Table 2. There were no discordant IDs. There were a total of 4 microorganisms where no ID was obtained using the LC; 2 at <1hr (*S. epidermidis*, and Strep), 1 at 3hr (Strep) and 1 at 5hr (Strep). One very major error (VME) was observed with *S. aureus* and penicillin at Time <1 hr. There were no major errors (ME) and 8 minor errors (mE) as shown in Table 2.

## RESULTS

### Table 1. Microorganisms Tested (bioMérieux Culture Bottles)

| Gram-Positive | Streptococcus | Gram-Negative | |
|---|---|---|---|
| *Enterococcus faecalis* | *Streptococcus agalactiae* | *Acinetobacter baumannii* | *Serratia marcescens* |
| *Enterococcus faecium* | *Streptococcus pneumoniae* | *Enterobacter cloacae* Complex | *Proteus mirabilis* |
| *Staphylococcus aureus* | *Streptococcus mitis/oralis* | *Escherichia coli* | *Citrobacter freundii* |
| *Staphylocoocus epidermidis* | *Streptococcus sanguinis* | *Klebsiella pneumoniae* | *Stenotrophomonas maltophilia* |
| | | *Pseudomonas aeruginosa* | |

### Table 2. Liquid Colony Stability at Time <1 hr RT, 3 hrs RT and 5 hrs RT

| Microorganism | Time in Hours LC Held @RT | ID Obtained (# replicates) | AST CA | AST EA | mE | ME | VME |
|---|---|---|---|---|---|---|---|
| Gram-positive (n=4) GP67 Card | 0 | 3/4 | 96.8% | 98.1% | 1 | 0 | 1* |
| | 3 | 4/4 | 100% | 100% | 0 | 0 | 0 |
| | 5 | 4/4 | 98.4% | 100% | 1 | 0 | 0 |
| Streptococcus (n=4) ST03 Card | 0 | 3/4 | 100% | 100% | 0 | 0 | 0 |
| | 3 | 3/4 | 100% | 100% | 0 | 0 | 0 |
| | 5 | 3/4 | 100% | 100% | 0 | 0 | 0 |
| Gram-negative (n=8) N392 Card | 0 | 9/9 | 97.5% | 98.3% | 3 | 0 | 0 |
| | 3 | 9/9 | 98.3% | 98.3% | 1 | 0 | 0 |
| | 5 | 9/9 | 99.2% | 99.2% | 2 | 0 | 0 |

VME = Very Major Error, ME = Major Error, mE = Minor Error

**S. aureus* and benzylpenicillin

## CONCLUSIONS

- ID using the LC was comparable with SOC at each time point however the identification of 1 strain of Streptococcus was missed at each time point.
- AST using the LC was comparable with SOC at each time point. Only 1 VME was observed in this study.
- This feasibility data support the potential role of the FAST-PBC Prep™ System in providing a LC directly from a PBC for use in automated ID/AST systems.
- Extended LC stability may be useful in laboratories where the LC cannot be used immediately after preparation. Each laboratory would need to perform their own verification on the stability of the LC before implementation of extended stability in their laboratory.

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