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Rapid Detection of *Enterobacteriaceae* Direct From Blood on Simulated Bacteremia Samples Using the Accelerate Pheno™ System

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Amended Abstract

Background: Rapid detection and identification of bacteremia directly from whole blood samples presents an ultimate challenge in blood-borne infection diagnostics. Current methods may take up to 48-72 hours to produce results, negatively affecting empiric therapy outcome. The purpose of this study was to assess sensitivity of a new rapid method using the Accelerate Pheno™ system with whole blood simulated *Enterobacteriaceae* bacteremia samples.

Methods: Cultures of *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, or *E. aerogenes* ATCC 13048 were inoculated into 10 mL of healthy donor blood to achieve initial sample concentrations ranging from 0.3 to 94 CFU/mL. Rapid blood sample processing included a short, 3-hour amplification step in a lytic growth medium followed by clean up and concentration steps. Each final sample was loaded onto the Accelerate Pheno™ system for analysis. The assay was designed for bacterial growth detection as well as limited identification (ID). Microbial cells in ID channels of the cassette were stained with either propidium iodide (universal), species-specific, or combination (*E. coli*, *Klebsiella* spp., and *Enterobacter* spp.) FISH probes. Images were analyzed both with a proprietary software program and manually. Concurrent quantitative plating provided data on initial and final sample concentrations.

Results: A total of 67 simulated bacteremia samples were processed using the developed method and analyzed with the Accelerate Pheno™ system. Experiments were performed with *E. coli* ATCC 25922 (35.8%), *K. pneumoniae* ATCC 700603 (29.9%), and *E. aerogenes* ATCC 13048 (34.3%). For all samples, growth was detected on TSA II 5% SB and on the Accelerate Pheno™ system following the rapid sample processing protocol. Growing microbial cells were observed in growth channels; stained single cells and cell clusters were clearly visible by fluorescence in ID channels.

Quantitative plating results showed that with an average initial simulated bacteremia sample concentration of less than 1 CFU/mL in 10 mL of blood, protocol yielded an average final sample concentration greater than $1x10^3$ CFU/mL, which was sufficient for manual growth detection.

Conclusions: This new rapid method demonstrated 100% detectability using the Accelerate Pheno™ system and whole blood simulated bacteremia samples with initial *Enterobacteriaceae* concentrations as low as 0.3 CFU/mL. The method has potential to significantly reduce time-to-result of ID/AST in direct from blood samples of bacteremic patients.

Methods

❖ **Experiment Steps:**

Spike donor whole blood with known concentration of *Enterobacteriaceae* isolate

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Perform rapid blood sample processing – 4 hrs

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Load sample onto Accelerate Pheno™ system for automated assay (**Table 1**) – 5 hrs

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Analyze images for detection and growth of microbial cells (**Images 1-4**) - Manual

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Confirm initial and final sample concentrations by quantitative plating (**Table 2**)

Results

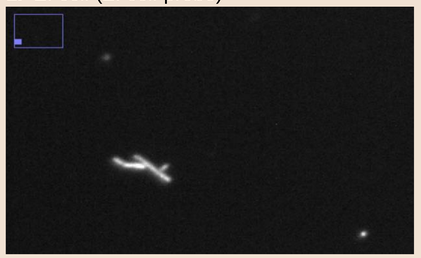
❖ **Number of Replicates per Experiment**

Table 1

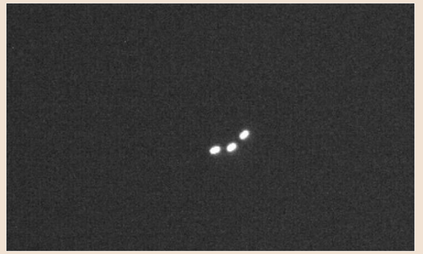
Stain or FISH Probe	Bacterial strains used for simulation			
	E. coli	*K. pneumoniae*	*E. aerogenes*	Total
PI (Universal)	6	8	14	28
E. coli	14			14
Klebsiella spp.		10		10
Enterobacter spp.			7	7
Combo (*E. coli, Klebsiella* spp., *Enterobacter* spp.)	4	2	2	8
Total	24	20	23	67

❖ **Fluorescent Images on the Accelerate Pheno™ system**

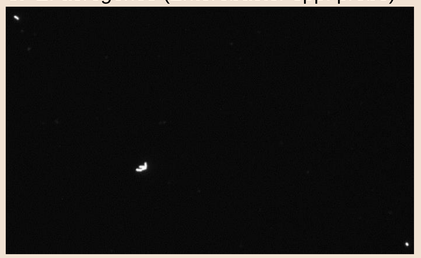
1. *E. coli* (*E. coli* probe)



2. *K. pneumoniae* (*Klebsiella* spp. probe)



3. *E. aerogenes* (*Enterobacter* spp. probe)



4. *E. aerogenes* (growth in dark field)

Results

❖ **Concentration Before and After Rapid Sample Processing**

Table 2

Organism	Number of Experiments	Initial CFU/mL in 10 mL blood	Final CFU/mL in 10 mL blood	Average Gain
E. coli	18	$5.8x10^{-1}$	$2.05x10^3$	3527-fold
	6	$7.8x10^1$	$2.79x10^5$	3582-fold
K. pneumoniae	17	$7.2x10^{-1}$	$3.48x10^3$	4832-fold
	1	$8.8x10^0$	$1.66x10^4$	1881-fold
	2	$6.0x10^1$	$5.45x10^5$	9083-fold
E. aerogenes	19	$6.9x10^{-1}$	$5.75x10^3$	8333-fold
	4	$6.8x10^1$	$7.24x10^5$	10731-fold

❖ **Amplification:** Quantitative plating after the rapid blood sample processing method showed an average of 3,527 to 10,731-fold growth (**Table 2),** producing sufficient quantity of microbial cells to be reliably detected by fluorescence during microscopic imaging.

<1 CFU/mL → **>5000 CFU/mL**

Limitations

❖ **Automated Analysis:** The images were analyzed manually in order to detect bacterial cells. There is no automated analysis built for this method to date, which means detection is quite time-consuming.

Conclusions

❖ **Detection** was achieved with this method of rapid blood sample processing followed by fluorescent staining and microscopic imaging for starting concentrations of bacteria ranging from 0.3 to 94 CFU/mL in simulated bacteremic patient blood with three different *Enterobacteriaceae* strains.

❖ **Rapid detection** and identification of blood-borne pathogens, coupled with rapid AST results, will enable clinicians to provide appropriate and timely antibiotic treatment to their patients in hours instead of days.

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