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A Case Report of *E. coli* Bacteremia Detection Direct from a Whole Blood Patient Sample Using the Accelerate Pheno™ System

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Amended Abstract

Background: *Blood culture remains the gold standard for diagnosing bloodstream infections in clinical microbiology. However, positive blood cultures (PBC) require further processing in order to obtain ID/AST results. A new method with capacity to be developed into a fully automated diagnostic assay was used to detect bacteremia directly from a whole blood patient sample.*

Methods: *A patient with confirmed E. coli bacteremia was identified as eligible for the study under approved COMIRB protocol and informed consent obtained. Within 24 h of the qualifying PBC, 20 mL of the patient's blood was collected with 10 mL inoculated into a BACTEC™ Plus Aerobic/F Culture Vial and 10 mL transferred into a BD SPS Vacutainer® blood collection tube. The BACTEC™ vial was incubated following BACTEC™ standard protocol (BC). The SPS tube sample was transferred into a 50 mL tube with lytic growth medium, incubated at 35˚C for 3 h, concentrated down to 1.5 mL and cleaned with various buffers (cleaned sample). 1.4 mL of the cleaned sample was tested on the Accelerate Pheno™ system with an investigational kit, and experiment images were analyzed both with a proprietary software program and manually. 100 uL of cleaned sample was plated onto TSA II 5% SB and incubated at 35˚C overnight (o/n). Resulting colonies were counted and used to calculate the number of CFU in the SPS tube (presuming an E. coli division rate of 2.4 div/h at 35˚C and 1.2 div/h at RT) and subcultured onto TSA II 5% SB. This o/n culture was inoculated into a BACTEC™ vial with 10 mL of human whole blood to mimic a human blood sample then incubated as previously described (seeded BC). At positivity, the seeded BC was run on the Accelerate Pheno™ system with an Accelerate PhenoTest™ BC kit. ID/AST results were compared to clinical lab results.*

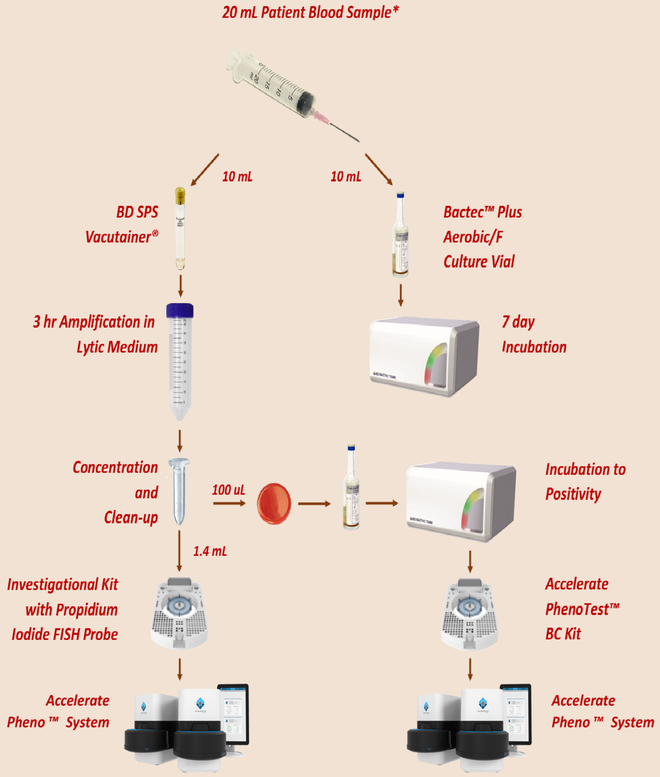
Results: *No growth was detected in the BC after 7 days incubation. Manual image analysis of the cleaned sample run on the Accelerate Pheno™ system resulted in detection of 2 rod shaped bacterial cells. Three (3) colonies, identical in morphology with a Gram stain of Gram negative rods, grew on TSA II 5% SB, corresponding to 30 CFU/mL, or 45 microbial cells in the final cleaned sample and 1.03 CFU in the SPS tube. Testing of the seeded BC resulted in ID of E. coli with 92.3% categorical agreement of its antibiotic profile with clinical lab results (12/13, 1 minor error).*

Conclusions: *The new method identified microbial growth direct from whole blood. Future development will incorporate detection and ID/AST in one assay, reducing time to result from days to hours.*

Introduction

A whole blood patient sample was processed using a new method for bacteremia detection and the Accelerate Pheno™ system.

Methods



**The patient had been administered antibiotics prior to the blood draw as follows: Azithromycin, 2 doses of 500 mg, and Ceftriaxone, 1 dose of 1 g.*

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Results

- No growth was detected in the BC after 7 days incubation.
- Analysis of a FISH channel plot, generated from proprietary software data outputs, and manual image inspection of the cleaned sample experiment on the Accelerate Pheno™ system resulted in detection of 2 rod shaped bacterial cells (Figures 1 and 2).
- Three (3) colonies, identical in morphology with a Gram stain of Gram negative rods, grew on TSA II 5% SB, corresponding to 30 CFU/mL, or 45 microbial cells in the final cleaned sample and 1.03 CFU in the SPS tube.
- Testing of the seeded BC on the Accelerate Pheno™ system resulted in ID of *E. coli* with 92.3% categorical agreement of its susceptibility profile with the clinical microbiology laboratory results (12/13, 1 minor error) (Table 1).

Figure 1. FISH Channel Plot

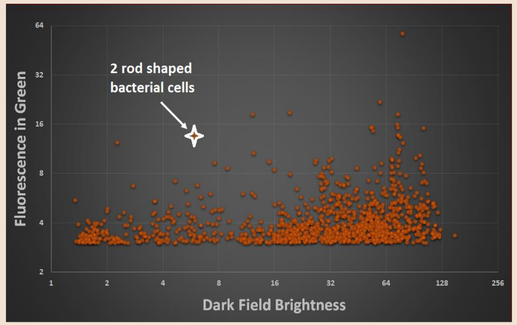

Figure 2. Dark Field Image of 2 Rod Shaped Bacterial Cells

Table 1. Clinical vs. Research Isolate Susceptibility Profiles

Antimicrobial	Clinical Microbiology Laboratory AST Results, SIR	Accelerate Pheno™ AST Results, SIR
Amikacin	Susceptible	Susceptible
Ampicillin-Sulbactam	Resistant	Resistant
Aztreonam	Susceptible	Susceptible
Cefazolin	Resistant	Resistant
Cefepime	Susceptible	Susceptible
Ceftazidime	Susceptible	Susceptible
Ceftriaxone	Susceptible	Susceptible
Ciprofloxacin	Susceptible	Susceptible
Ertapenem	Susceptible	Susceptible
Gentamycin	Susceptible	Susceptible
Meropenem	Susceptible	Susceptible
Piperacillin-Tazobactam	*Intermediate*	*Susceptible*
Tobramycin	Susceptible	Susceptible

Conclusions

The new method identified microbial growth directly from a whole blood patient sample. Incorporation of detection and ID/AST into one assay will significantly reduce time to result from days to hours.