P0539
Poster Session II
Molecular diagnosis of bloodstream infections

PATHOGEN IDENTIFICATION FROM POSITIVE BLOOD CULTURES USING AUTOMATED SAMPLE PREPARATION AND AUTOMATED FLUORESCENT IN SITU HYBRIDIZATION (FISH)

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Objectives: The purpose of this study was to characterize a novel automated sample preparation method with positive blood cultures, and subsequent identification using multiplexed fluorescent *in situ* hybridization (FISH) using known isolates.

Methods: A total of 205 known isolates were evaluated in this study; 56 Gram-positives (16 *Enterococcus* spp., 40 *Streptococcus* spp.), 42 Gram-negatives (12 *Escherichia coli*, 7 *Serratia marcescens*, 15 *Proteus* spp., and 8 *Pseudomonas aeruginosa*) and 107 non-targets. Each isolate was separately seeded into simulated blood culture bottles (1 part of healthy donor blood + 4 parts of BD BACTEC Standard Aerobic media) and incubated overnight for approximately 20-24 h. Culture aliquots were then subjected to an automated gel electrofiltration preparation procedure for 20 min in a pipetting robot. 200 µL aliquots of each prepared culture sample were manually diluted to approximately 5 x 10^5 to 5 x 10^6 CFU/mL in an electrokinetic buffer, then used to inoculate a series of independent flowcell channels in a 32-channel disposable fluidic cassette for bacterial immobilization. Immobilized bacteria were permeabilized, and each flowcell received one of 10 unique cocktails of ATTO-550 fluorescently labeled FISH probes for targeted species groups. The bacteria were then washed under stringent conditions and the fluorescence intensity of labeled bacteria was measured using automated epi-fluorescence microscopy. An ATTO-647-labelled eubacteria probe was run in parallel in every channel. The eubacteria signal level was compared to the target probe levels to differentiate debris from bacterial cells. An automated image processing algorithm counted labeled bacterial cells in the microscopy images for each flowcell.

Results: The FISH method agreed with a known isolate identity in 203 of 205 tests (100% sensitivity and 98% specificity).

Conclusion: The novel automated sample preparation and multiplexed FISH identification methods produced accurate sample-to-answer results for 10 bacteremia-associated species of Gram-positive or Gram-negative bacteria. Automated image acquisition and processing were used to analyze up to 32 samples in a single multiplexed FISH analysis. These results justify further studies with additional pathogenic species and integration into automated diagnostic systems.