Rapid Bacterial Identification Directly from Positive Blood Cultures
Using Automated Sample Preparation and Multiplexed Fluorescence in situ Hybridization (FISH)

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AMENDED ABSTRACT
Background: Rapid bacterial identification is critical in guiding appropriate antibiotic therapy, preventing morbidity and mortality in patients with sepsis. The current gold standard for identification of bloodstream pathogens is manual interpretation. This method could be used to rapidly identify bloodstream pathogens, limiting the procedure to high concentration samples. Adding a sample pretreatment step allows automated analysis of low concentration samples such as respiratory and urinary tract specimens.

RESULTS
1. Each sample (20 μL) was pipetted into individual flowcell chambers of 32-channel disposable cassette. A 5×10^6 to 5×10^7 CFU/mL in L-DOPA buffer.
2. A pipetting robot performed sample cleanup using automated gel electrophoresis. Each flowcell was rinsed twice with wash buffer (0.01% SDS, 50 mM EDTA, 200 mM NaCl) to remove fluorescent debris in this analysis. EUB338 labeled probes (15, 8, 10, 12, 13, 15, 17, 19, 21, 23, 25, 26, 27, 29, 31, 33, 39, 43, 49, 57, 67, 71, 73, 79, 91, 97, 109, 127, 133).
3. Automated data analysis was performed by treatment with peptidoglycan-targeting enzymes for 6 min followed by high-voltage electric field immobilized bacteria on a disposable cassette. A 5-min low-voltage electric field immobilized bacteria on a disposable cassette. A 5-min low-voltage electric field immobilized bacteria on a disposable cassette.
4. Images were taken in 3 fields of view (Figure 2) for 1 s per field of view at 550 nm and 647 nm in dark-field.

Figure 1: Process flow including simulated blood culture preparation, sample cleanup by automated gel electrophoresis, automated transfer to the cassette for bacterial identification by electronic concentration, FISH automated imaging, and automated data analysis.

Figure 2: Examples of images and automated analysis histograms of bacterial cell fluorescence (15, 8, 10, 12, 13, 15, 17, 19, 21, 23, 25, 26, 27, 29, 31, 33, 39, 43, 49, 57, 67, 71, 73, 79, 91, 97, 109, 127, 133).

Figure 3: Representative images and signal-to-background (S/B) ratio histograms of a K. pneumoniae sample in two different fields shown at 100×. Sample concentration in dark-field acquired images in dark-field and 647 nm (fluorescent bacterial probe and target probe probes). Universal bacterial probe is fixed to target probe probe. Universal bacterial probe is fixed to target probe probe.

Each fluorescent object was referenced to its matching dark-field object to eliminate debris in this analysis. EUB338 labeled probes (15, 8, 10, 12, 13, 15, 17, 19, 21, 23, 25, 26, 27, 29, 31, 33, 39, 43, 49, 57, 67, 71, 73, 79, 91, 97, 109, 127, 133).

The FIG method allowed accurate bacterial identification at varying concentrations.

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