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Same-Day Blood Culture with Digital Microscopy

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AMENDED ABSTRACT

Background: Critically ill patients who acquire a bloodstream infection must begin adequate antibiotic therapy as quickly as possible. The requirement for overnight culture creates an unacceptable delay. Automated microscopy offers the potential for same-day turnaround including organism identification and drug resistance phenotype characterization.

Methods: 29 aliquots of 10 mL each were taken from two short-fill CPD blood bank bags. Each sample received isolate spikes to make nominal 5 CFU/mL of bacterial target species. These included 14 *Staphylococcus aureus* (SA), and 3 *Pseudomonas aeruginosa* (PA) plus 12 non-target Gram-negative bacilli. Each sample was diluted 4-fold to promote growth. 35°C incubation for 4 hours was followed by centrifugation with final resuspension in 1 mL of electrokinetic buffer. 16 flowcell channels in a multichannel fluidic cassette each received 20 µL of sample, followed by 5-minute electrokinetic concentration and surface capture. Liquid (40° C) Mueller-Hinton agar with and without antimicrobials was then exchanged through each channel and gelled. Automated microscopy acquired images at 10-minute intervals for 3 hours. Image analysis detected clonal growth, identified SA and PA, and simultaneously performed resistance phenotype tests using 32 µg/mL amikacin, 8 µg/mL imipenem, 6 µg/mL cefoxitin, or 0.5 µg/mL clindamycin. Controls included quantitative culturing, disk diffusion tests for isolate resistance phenotype, and 20 blood samples without spikes.

Results: Median input concentration was 5 CFU/mL. Culture confirmed that normal growth occurred in the prepared samples. Microscopy detected growth in 29/29 spiked samples and none in 20/20 controls. Enough organisms grew in 4 hrs. for ID tests in 23/29 samples, with 1/1 PA and 10/10 SA identified. Of 22 non-targets, 1 false-positive PA ID error occurred. Concordant resistance was reported for 19/20 tests (1 false MSSA). Combined ID sensitivity and specificity were 100% and 97%, and phenotyping was 89% and 100%. Total run time was 8 hours from start to finish. Post-incubation organism detection required ≥4 growing clones (GC). ID required ≥40 GC, and each phenotype test required ≥40 GC.

Conclusions: Automated microscopy demonstrates promise for same-day pathogen identification and drug resistance phenotype detection directly from blood.

INTRODUCTION

Bacteremia due to multiple drug resistant organisms (MDRO) is increasing in frequency and growing in complexity. For critically ill patients, resistance can render initial therapy ineffective, delaying the start of effective antimicrobial therapy. Delay also prolongs exposure to broad-spectrum empiric therapy, creating selective pressure favoring emergent resistance. Multiplexed automated digital microscopy (MADM) has the potential to reduce turnaround time by rapidly analyzing live bacteria extracted directly from a clinical specimen, eliminating the need for colony isolates. The purpose of this pilot study was to determine MADM sensitivity, specificity, speed, and technical requirements for same-day analysis of live organisms extracted directly from blood. Tests used two of the most common ICU pathogens, *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA).

MATERIALS & METHODS

The MADM system used a custom microscope and pipetting robot, plus custom image analysis and experiment control software. 32-channel disposable cassettes (**Fig. 1**) enabled live microbial cell immobilization for microscopy and fluid exchanges for different test agents. The microscope scanned 40 image fields in each flowcell channel, each channel having organisms extracted from about 3.5 µL of prepared inoculum.

Figure 1a: *32-channel flowcell cassette.*

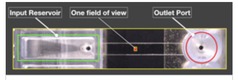

Figure 1b: *One flowcell channel.*

Simulated blood specimens consisted of isolates spiked into 10 mL each of 29 aliquots of banked blood to make approximately 5 CFU/mL, confirmed by quantitative culture. Spiked isolates included 14 *Staphylococcus aureus* (SA), 3 *Pseudomonas aeruginosa* (PA), or 12 non-target species. Dilution of each sample with 30 mL of modified TSB culture medium promoted growth. 20 additional control aliquots contained no spikes. 4-hour incubation at 35ºC, followed with brief spin cleanup, ended with pellet resuspension into an electrokinetic buffer to make 1 mL. 20 µL sample aliquots were then pipetted into 14 cassette flowcells (**Fig. 2**). A 5-minute low-voltage electrical field concentrated bacteria to the lower surface of each flowcell where a capture coating immobilized the bacterial cells.

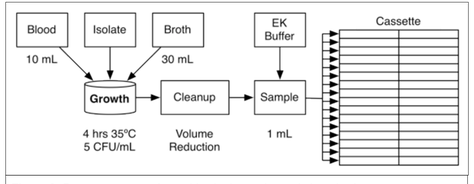

Figure 2: *Process summary for rapid culturing and sample introduction.*

The system acquired dark-field images every 10 minutes. The analyzer applied identification algorithms to each individual immobilized cell that exhibited growth. 6 channels provided data for ID algorithms to score individual organisms and their progeny clones. ID variables included cell morphology, clone growth morphology, clone growth rate, and other factors. The analyzer computed ID probability based on the number of related clones and their scores. The system required 40 or more clones that exceeded a threshold score in order to proceed with analysis.

Separate pairs of channels received antibiotics in 40ºC liquified Mueller-Hinton agar: 32 µg/mL amikacin (AMK), 8 µg/mL imipenem (IPM), 6 µg/mL cefoxitin (FOX), or 0.5 µg/mL clindamycin (CLI). Cooling then gelled the agar, followed by incubation at 35ºC with microscope imaging at 10-minute intervals for 3 hours (concurrent with identification in other channels).

Comparators included quantitative cultures, and disk diffusion phenotype tests for drug resistance.

RESULTS

Organism detection required ≥4 growing clones (GC). Recovery yielded SA GC counts that exceeded CFU as determined by culturing because of near-complete clump disruption in most samples. Counting combined results in multiple channels when appropriate. Identification required ≥40 GC, and each phenotype test required ≥40 GC. MADM detected growth in 29/29 spiked samples and no growth in 20/20 non-spiked controls. Growth sufficient for ID occurred in 23/29 samples. 4 SA samples clumped excessively, precluding ID scoring. 2 PA samples grew too slowly (<1.1 div/hr) to achieve 40 GC in the fixed 4-hour growth period (5 hours would suffice). SA growth rates were ≥1.5 div/hr. MADM identified 1/1 PA and 10/10 SA. One false PA ID occurred out of 22 non-target samples to yield 100% sensitivity and 97% specificity. The false ID was attributable to a known imaging aberration, later corrected.

Fig. 3 shows examples of dark-field images for 3 hours of clone growth for SA without drug, SA in 6 µg/mL FOX, and for a Gram-negative rod (*E. coli*) without drug for morphology comparison.

MADM reported drug resistance in 19/20 adequate samples with one false MSSA, yielding 89% sensitivity and 100% specificity. The table summarizes SA data for overall concordance with comparator results.

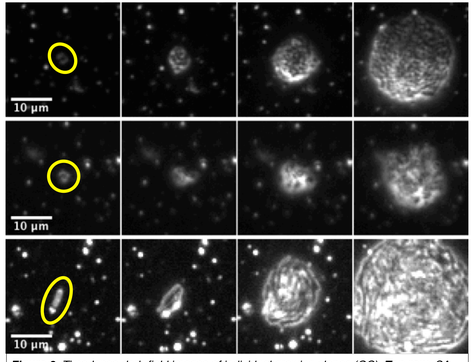

Figure 3: *Time-lapse dark-field images of individual growing clones (GC).* ***Top row:*** *SA growth control (no antibiotic) at 0, 60, 120, and 180 minutes. Small cell cluster as the starting CFU (yellow ellipse).* ***Middle row:*** *SA in 6 µg/mL cefoxitin, growth indicating MRSA phenotype. Larger starting CFU than the growth control but slower growth.* ***Bottom row:*** *E. coli growth control to illustrate obvious morphological and growth rate differences. Brighter areas represent 3-dimensional growth effect (more light scattering from layering or end-on rod orientation). Non-growing particles are assumed to be debris.*

DISCUSSION

This pilot study asked whether major pathogens grow quickly enough to enable same-day diagnostic testing directly with bacteremic blood samples using microscopy. MADM had previously analyzed small numbers of live microbial cells extracted from other specimen types. This study demonstrated that 4 hours of growth in a common nutrient medium provides enough live clones for MADM analysis with fast-growing cells (>1.1 div/hour growth rate in the conditions tested). PA required slightly longer times for adequate testing, estimated at 5 hours. These results provide parameters for determining requirements for practical application. Given the number of GC required for a test (40 with the study prototype), number of tests, and the slowest target organism growth, straightforward calculation derives the minimum growth duration needed. Fastest possible turnaround time results from maximizing growth rate while minimizing the GC needed per test, and minimizing the required number of tests and their duration.

IDENTIFICATION (Adequate Growth N=23)			
S. aureus	**True Neg**	**True Pos**	**Accuracy**
MADM-Pos	0	**10**	Sens 100% (CI 66-100%)
MADM-Neg	23	0	Spec 100% (CI 72-100%)
PHENOTYPE: MRSA (Adequate Growth N=10)			
MADM-Pos	0	4	Sens 80% (CI 30-100%)
MADM-Neg	5	1	Spec 100% (CI 46-100%)
PHENOTYPE: CLI-R (Adequate Growth N=10)			
MADM-Pos	0	4	Sens 100% (CI 40-100%)
MADM-Neg	6	0	Spec 100% (CI 52-100%)

Within 8 hours starting with blood, automated microscopy successfully identified target pathogens and detected drug resistance phenotypes for a major species of live bacterial cells extracted directly from a small volume of simulated bacteremic blood. Diagnostic analysis using individual live-cell methods enables rapid turnaround without first requiring colony isolates. The probabilistic identification scoring achieved high concordance with clinical lab results. Resistance phenotype analysis also achieved high concordance. This analytical strategy can also use responses of individual clones to identify organism subpopulations and resistance phenotypes within polymicrobial specimens.

Conclusions: MADM enables diagnostic analysis of live microbial cells extracted after brief growth in culture medium. The next requirement is for proof of concept using a broadened panel of prevalent organisms and their critical drug resistance phenotypes. This study identified operational variables and parameters needed to achieve this objective.