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Same-Shift ID Directly from Respiratory Specimens by Automated Microscopy

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AMENDED ABSTRACT

Background: Rapid, same-shift, quantification and ID (qID) of multiple drug resistant (MDR) *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) in lower respiratory tract infections (LRTI) could reduce clinical morbidity and mortality.

Methods: A clinical pilot study compared culture with multiplexed automated digital microscopy (MADM) using LRTI specimens. 59 de-identified remnant scoped bronchial alveolar lavage (BAL) specimens were collected from July 2010 to March 2011 at Barnes-Jewish Hospital (St. Louis, MO) and selected for the study. Study culture, consisting of 1 µL loop-based semi-quantitative plating (SQP) and identification, was performed on the same day that 0.5 mL of the remnant was run on the MADM system. MADM monitored growth of viable single bacteria, enabling quantitative ID for SA and PA. Culture and MADM results were retrospectively compared.

Results: 53 of the 59 specimens had successful MADM tests. By SQP, 16 of the 53 were above diagnostic threshold (1x10⁴ CFU/mL) for SA (n=9) or PA (n=7). MADM identification was 100% concordant with these samples. With 2 of the PA and 1 SA specimens, MADM identified less than (while SQP detected more than) the diagnostic threshold of the target organisms. By SQP, 46 of the samples did not contain PA above the diagnostic threshold. By MADM, 1 specimen was false-positive for PA resulting from a technical failure (out-of-focus imaging). Additionally, 1 specimen contained greater than (while SQP detected less than) the diagnostic threshold of PA. MADM was 96% (44/46) specific for PA quantitation. By SQP, 44 of the samples did not contain SA above the diagnostic threshold. By MADM, 2 specimens contained greater than (while SQP detected less than) the diagnostic threshold of SA. MADM specificity was 95% (42/44) for SA quantitation. The MADM time from specimen to result was 1 hour for specimen cleanup plus 3 hours for extended growth data.

Conclusions: MADM ID was concordant with culture and highly specific. Discordant quantitation is explained by high variance of the semi-quantitative loop culture comparator. MADM appears promising for same-shift qID directly from LRTI specimens.

INTRODUCTION

Nosocomial infections due to multiple drug resistant organisms (MDRO) are increasing in frequency and growing in complexity. For critically ill patients, resistance can render initial therapy ineffective, delaying the start of effective antimicrobial therapy. Multiplexed automated digital microscopy (MADM) has the potential to reduce turnaround time by rapidly analyzing bacteria extracted directly from a clinical specimen, eliminating the need for enrichment or isolate preparation. The purpose of our study was to determine MADM sensitivity, specificity, and speed for identifying individual live organisms extracted directly from specimens, and to assess quantitation accuracy in measuring specimen concentrations of each targeted organism using culture methods as the comparators. Tests targets were the two most common pneumonic ICU pathogens, *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA).

MATERIALS & METHODS

MADM systems used commercial microscopes and accessories, plus custom image analysis and control software. 32-channel disposable cassettes (Fig. 1) enabled live microbial cell immobilization for microscopy and pipette exchanges for different test fluids in Mueller-Hinton broth. The microscope scanned 40 fields in each flowcell channel, each channel having organisms extracted from about 3.5 µL of prepared inoculum (112 µL per cassette).

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

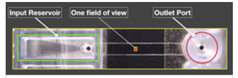

Figure 1b: *One flowcell channel.*

Extraction of live bacteria consisted of sample cleanup (Fig. 2) followed by introduction of the sample into the cassette's fluidic channels. A 5-minute low-voltage electrical field concentrated bacteria to the lower surface of each flowcell where a capture coating immobilized the bacterial cells. Following a brief wash, the system then acquired dark-field images every 10 minutes. The system applied ID algorithms to each individual immobilized cell that exhibited growth.

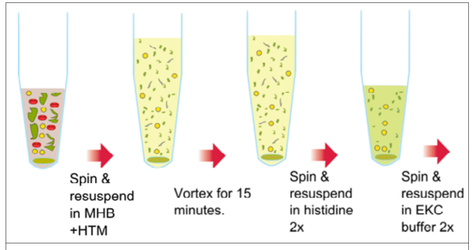

Figure 2: *Specimen cleanup.*

Image analysis to ID individual organisms and their progeny clones included variables derived for cell morphology, clone growth morphology, clone growth rate, and other factors. The analyzer computed ID probability based on the number of clones with significant scores. The system required 20 or more clones to exceed a scoring threshold in order to report an ID. This study used 3 channels per specimen, with a lower limit of quantitation of 2x10³ CFU/mL and a readable range of about 3.5 logs (max. 2x10⁷ CFU/mL per channel) for BAL specimens prepared by the described method.

Quantitation consisted of counting identified, growing clones and computing original specimen CFU/mL using the total concentration and recovery factors from inoculum preparation. MADM algorithms treat one growing clone (GC) as equivalent to one CFU.

We used 0.5 mL aliquots of 63 de-identified BAL specimens acquired from ICU patients and known from clinical lab reports to contain SA, PA, or other organisms with ≥10⁴ CFU/mL. An Institutional Review Board approved the study.

As a quantitation and viability control, we used a concurrent 1 µL semi-quantitative loop culture of an aliquot of each MADM sample. The control procedure was identical to that used for the clinical lab result.

RESULTS

Of 63 specimens, 4 had discordant results on repeat culture (no growth in the control culture). Six MADM runs had technical failures related to operator error or instrument deficiencies, leaving 53 valid runs.

Specimen cleanup removed substantial debris, enabling high recovery and automated individual clone tracking with MADM. 16 culture-positive specimens contained SA (9) or PA (7). The remaining 37 specimens contained non-target organisms or were control negative. Target-positive specimens often contained non-target organisms. None contained both SA and PA above diagnostic threshold.

The MADM system correctly identified the organisms in all target-positive specimens (9/9 SA and 7/7 PA). The system reliably tracked growing progeny clones against complex background, imposing a growth requirement to mask non-growing debris. Growth sufficient for analysis occurred in 30-90 minutes, depending on organism growth rate and the time that differentiating variables emerged. In order to derive optimization data, experiment duration in this study exceeded the minimum required.

SA appeared as relatively bright cocci in dark field (Fig 4a). PA appeared as dim, thin rods (Fig. 4b) in dark field. Note the non-growing background debris in the PA figure, which the image analyzer ignores.

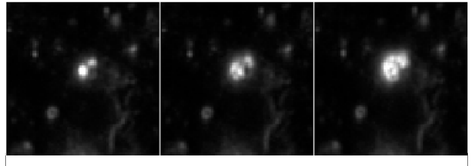

Figure 4a: *Adjacent pair of SA growing clones at 0, 40, and 80 minutes after capture.*

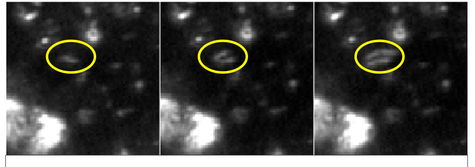

Figure 4b: *PA growing clone at 0, 40, and 80 minutes after capture.*

Positive specimens had target organism content between 1x10⁴ (diagnostic threshold), 2.3x10⁶ (SA), and 1.1x10⁶ (PA) CFU/mL by MADM counts. Although the semi-quantitative loop culture method has high variance and MADM has much lower variance, the mean absolute difference was only 0.03 logs. 1 µL loop culture has an effective limit of quantitation of about 2x10⁴ CFU/mL—above the diagnostic threshold.

8 specimens contained target organisms below diagnostic threshold and above 10³ CFU/mL as determined by the control culture, but were ruled positive by the previous clinical culture. Of these, MADM analysis supplemented with manual audit correctly identified the organisms. MADM, however, measured one PA- and one SA- containing specimen as above diagnostic threshold (1.7 and 2.1x10⁴, respectively) by the automated method alone. The table includes these in its summary of overall quantitation concordance for positive/negative calls.

QUANTITATION CONCORDANCE

		MADM Agree	MADM Disagree: Loop	MADM Disagree: MADM
SA	Loop Pos	8/9	1.3x10⁴	4.5x10³
	Loop Neg	42/44	2.0x10³	2.1x10⁴
			2.0x10²	1.6x10⁴
PA	Loop Pos	5/7	1.0x10⁴	2.2x10³
			2.6x10⁴	3.9x10³
	Loop Neg	44/46	4.0x10³	1.7x10⁴
			0	3.2x10⁴

DISCUSSION

Automated microscopy enabled successful identification and quantitation for two major species of live bacterial cells extracted directly from BAL specimens and achieving adequate ID scores in less than 2½ hours. The 6x10³ CFU/mL lower limit of quantitation offers a safe margin for identifying bacterial burden below the diagnostic threshold of 10⁴ CFU/mL for BAL.

Diagnostic and quantitative accuracy using cell-by-cell identification enables rapid analysis of polymicrobial specimens without first requiring physical isolation of different species. Sophisticated image analysis allowed prolonged tracking and precise growth rate measurement of individual clones as well as detailed morphology analysis. The probabilistic identification scoring achieved concordance with clinical lab results. This analytical strategy enables further testing using responses of individual clones within each subpopulation of identified organisms.

Fluid exchanges, image acquisition, and image analysis can be fully integrated and automated as a single instrument.