Rapid Quantitation and Identification of Pseudomonas aeruginosa, Staphylococcus aureus, and Acinetobacter baumannii in Bronchoalveolar Lavage Fluid S. Metzger1, C.S. Price2, W.M. Dunne, Jr.3, A. Shamshayeva4, K. Hance5, J. Mascali6, W. Kim6, D. Jolly7, I. Yushkevich8, K. Havens9, D. Howson1
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INTRODUCTION
Nosocomial infections due to multiple drug-resistant (MDR) bacteria are increasing in frequency and growing in complexity. For critically ill patients, resistance can render initial therapy ineffective, placing patients at risk for the development of effective antimicrobial therapy. But standard diagnostic cultures require 2-3 days to identify MDR organism pathogens and may result in empirical treatment of nosocomial resistance. In contrast, multiplexed automated digital microscopy (MADM; REMARK) has the potential to reduce turnaround time by rapidly identifying and characterizing these augmented identification methods for bronchoalveolar lavage fluid. Quantitation range and identification accuracy appear compatible with diagnostic requirements with an ongoing development, and are now approximately 30 minutes.

DISCUSSION
Growth sufficient for analysis occurred in 30-60 minutes. Labelling required less than 45 minutes using primary and secondary antibodies for a total analysis time of less than 2 hours. A 1:1 primary and secondary antibody conjugate was used. As performed in this study, sample preparation time was about 45 minutes. The preparation methods have been, however, largely reduced after ongoing development, and are now approximately 30 minutes. The small number of cells required is compatible with the bacterial concentration at BAL diagnostic threshold of 10⁵ CFU/mL. Accuracy using cell-by-cell identification is consistent with requirements for analysis of polymicrobial specimens. Additional research with pneumococcal pathogens using multiple evaluation methods for bronchoalveolar lavage fluid, Quantitation range and identification accuracy appear compatible with diagnostic requirements with an ongoing development, and are now approximately 30 minutes.

CONCLUSIONS
Multiplexed automated digital microscopy (MADM) identified and quantified two prevalent bacteria extracted using simple preparation methods for bronchoalveolar lavage fluid. Quantitation range and identification accuracy appear compatible with diagnostic requirements with an ongoing development, and are now approximately 30 minutes.

MATERIALS & METHODS
Customized MADM systems used commercial inverted microscopes with 32-channel disposable cassettes. A PC ran custom image analysis and experiment control software. Each 32-channel disposable cassettes (Fig. 1) enabled live microbial immobilization for microscopy and fluid exchanges for different test media and reagents.

We tested organism recovery, quantitation, and identification from BAL specimens by pooling de-identified bronchoscopic bronchoalveolar lavage (BAL) samples from ICU patients and spiking aliquots with culture broth containing each cultured isolate. Toxin test results were determined by a threshold of 0.5 McFarland turbidity. Isolates included 20 Pseudomonas aeruginosa (PA), Acinetobacter baumannii (AB), and Staphylococcus aureus (SA); plus 20 isolates of non-target pathogens commonly found in lower respiratory tract infections, including haemophilus influenzae. We also tested each individual original specimens (not spiked, not pooled). Controls used standard culturing methods.

Quantification by microscopy achieved a high log recovery, which is comparable to that of quantification in 30 minute culture plates. Susceptibilities for clonal counts had a higher sub-saturation distribution than culture broth due to the variation in numbers of clones used with microscopy (Fig. 3).

RESULT
A field of view typically contained 10 to 15 growing clones. An antibody labeling score. Specificity was, respectively, 90%/93% for SA, 95%/96% for PA, and 92%/98% for AB. The ROC curves (Fig. 5) show the morph and antibody scores combined to improve total identification accuracy (98%/100%).

All of the original BAL specimens examined separately, were positive for PA (above diagnostic threshold of 10⁵ CFU/mL). Other specimens were microbiologically negative. The microscopy results for positive and negative specimens matched those from standard culture methods.

REFERENCES

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