

## CONFIDENTIAL

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### Direct Detection and Enumeration of Viable Bacteria in Human Bronchial Washing Specimens Using Automated Growth Rate Analysis

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**Abstract:**

**Background:** Culturing substantially delays reporting to the physician who treats critically ill patients. To eliminate culturing prior to testing, it is necessary to extract viable bacteria directly from a specimen and present them in a form suitable for analysis. **Methods:** 27 random specimens of human bronchial washings were analyzed using automated microscopy for growth rate analysis and progeny clone enumeration of bacteria extracted and immobilized in a multi-channel flowcell device. An aliquot of each specimen was also analyzed by quantitative agar culture. Specimens ranged from cloudy, mucus-containing and blood-tainted to a clear water-like appearance. Specimens were treated with an erythrocyte lysis buffer and centrifuged on 1.03 g/mL Percoll® to separate bacteria from debris. Elapsed time was less than 45 minutes. Resuspended bacteria were delivered to the flowcells, electrokinetically concentrated, and immobilized on a poly-L-lysine-coated glass surface. The device incubated the cells at 35°C for 3 hours and acquired images every 10 minutes. Size and growth rate criteria were used to exclude yeasts and residual debris from analysis. Growing clones were enumerated and cell morphology was determined. Specimen isolates were Gram stained, and cell morphology was compared to those in acquired images.

**Results:** Quantitative cultures confirmed clinically relevant levels of bacteria ( $\geq 10^5$  CFU/mL) in 9 specimens. The experimental device detected growing clones in 8 of the specimens in less than 1 hour. Cell morphology of immobilized growing clones was consistent with that of Gram-stained cells. Morphology of immobilized non-growing cells was also consistent with the Gram-stained cells.

**Conclusions:** The automated system was able to enumerate diagnostic levels of growing bacteria extracted directly from bronchial washings in less than 2 hours after specimen access. The methods are consistent with the requirements for rapid diagnostic analysis of pathogens without prior culturing and isolation. Additional experimentation is required to determine the quantitative relationship between the experimental device and conventional methods.

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