1219 Rapid Host mRNA-Based Discrimination Between Bacterial and Viral Infection

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Background
Improving outcomes for patients with bacterial infection depends on early and accurate diagnosis followed by antibiotic treatment. Available diagnostic methods are too slow or limited in scope to meet these needs, leading to over-prescription of antibiotics and antimicrobial resistance. To address this, we have identified 7 host response mRNA biomarkers (HostDx™ Fever) that demonstrate an AUROC of 0.91-0.93 for discriminating bacterial from viral infection across independent cohorts. To measure these markers on a clinically relevant timescale, we have developed ultra-rapid proof-of-concept loop mediated isothermal amplification (LAMP) assays. We demonstrate quantitative expression analysis and verification of performance in patient samples.

Methods
LAMP primers were designed to amplify target mRNA and exclude DNA amplification by targeting primers to splice junctions. Solutions were identified for 3 biomarkers and one housekeeping gene. The quantitative dynamic range of the assays was determined using serially diluted control material to assess variance and linearity as a function of input. Relative abundance of targeted mRNA biomarkers in total RNA extracted from preserved patient blood samples was quantitatively measured and evaluated to determine concordance with an amplification-independent gold standard (NanoString nCounter).

Results
Primer solutions were iteratively optimized to achieve rapid turnaround times and mRNA specificity. All assays demonstrated a log-linear relationship to template input over a 6-log dynamic range, with a limit of quantitation around 103 copies. We measured the abundance of our markers in clinical samples using optimized assays and achieved a mean time to result of 12.2 minutes. HostDx Fever scores calculated based on LAMP and nCounter SPRINT measurements were well correlated (r = 0.93).

Conclusions
Accurate and rapid quantitation of mRNA expression levels across a dynamic range spanning at least 6 orders of magnitude is attainable with LAMP technology. The HostDx Fever LAMP assay panel is compatible with any platform capable of quantitative, time-resolved fluorescent detection at constant temperature and number of parallel reactions commensurate with the number of targeted biomarkers. This approach enables early and accurate diagnosis of acute infections and antibiotic prescription.