Isothermal amplification of host immune mRNAs for rapid bacterial/viral discrimination

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Background
Accurate diagnosis and treatment of bacterial infection is critical for improving patient outcomes. Severe adverse outcomes can be the result of both over-prescription (ie, C. difficile infections, adverse drug reactions and antimicrobial resistance) and under-prescription (increased morbidity and mortality). As culture-based pathogen detection is slow and molecular detection is limited in scope, an unmet need remains for a rapid test to differentiate between viral and bacterial infections. Using in-silico analyses across multiple independent cohorts we have previously published a 7-host mRNA signature, HostDx™ Fever, demonstrating an AUC of 0.93 for separating bacterial from viral infections using whole blood (Sweeney et al, Sci Transl Med, 2016). In recent prospective trials AUCs ranged between 0.88-0.96 for separating physician-adjudicated bacterial and viral infections in hospital settings. We here describe an ultra-rapid isothermal amplification solution designed to quantitate these mRNAs in rapid point of care testing.

Methods
Reverse transcription loop-mediated isothermal amplification (RT-LAMP) primers were designed for mRNA targets, with specificity enhanced by targeting FIP primers to splice junctions. Analytical and clinical accuracy was tested on RNA extracted from banked blood samples from patients with confirmed viral or bacterial infections. Quantitation in LAMP was compared to NanoString nCounter™.

Results
Iterative optimization of primer design resulted in RT-LAMP assays that selectively amplify target mRNA. Assays demonstrate a linear dynamic range spanning 6 orders of magnitude. Quantitation of relative expression levels for the targets of the HostDx Fever test showed good concordance ($R = 0.96$) with nCounter data in blood samples of 6 patients each with confirmed viral and bacterial infections as well as in 10 healthy controls. Bacterial and viral infections were perfectly discriminated. Average threshold times were <15 minutes.

Conclusion
Accurate discrimination of bacterial and viral infection was achieved on a point of care timescale using isothermal amplification. The assays could be run on any molecular diagnostic instrument that can measure 8 targets simultaneously. An IVD test distinguishing between bacterial and viral infections could aid in antimicrobial treatment decisions and improve clinical outcomes.