Multiplex RT-qPCR for diagnosis and risk stratification of acute infection and sepsis using a 30-mRNA host response signature

D. Rawling, PhD, M Remmel, MSc, W Nie, PhD, N Huynh, PhD, K Hecker, PhD, O Liesenfeld, MD, J Romanowsky, MBA, and TE Sweeney, MD, PhD.

Inflammatix Inc., Burlingame, CA 94010, USA

Background
Acute infections and sepsis, as leading causes of morbidity and mortality, represent a major burden to healthcare systems. Diagnostic procedures to evaluate patients with suspected acute infections or sepsis are inaccurate or slow. Using in-silico analysis of mRNA expression data from patients with acute infections and sepsis, we identified separate blood host-response signatures for the diagnosis and risk stratification of patients with acute infections or sepsis (Sweeney et al, Sci Transl Med, 2017; Sweeney et al, Nat Comms, 2018). Combined into a 30-host-mRNA panel called HostDx™ Sepsis, together with custom machine learning algorithms, the resulting test can identify (1) the presence of a bacterial infection, (2) the presence of a viral infection, and (3) the risk of 30-day mortality. High diagnostic and prognostic accuracy were upheld in prospective clinical studies. We here describe the development of a multiplex qPCR panel for rapid measurement of HostDx Sepsis.

Methods
To convert the gene set of the HostDx Sepsis test (29 targets plus 1 control) into rapid RT-qPCR assays, groups of 4- or 5-plex assays were designed. Total RNA was extracted (QIAcube™, QIAGEN®) from banked clinical PAXgene RNA blood samples (9 bacterial infections, 6 viral infections, 6 healthy controls). Multiplex TaqMan RT-qPCR assays were run on QuantStudio™ 6 (Thermo Fisher Scientific®) and compared to nCounter® SPRINT (NanoString®), an amplification-independent mRNA quantitation technology.

Results
Overall, multiplex RT-qPCR assays were highly concordant with NanoString results for all targets across all samples ($R > 0.95$). In this pilot study, bacterial and viral infections were perfectly separated (AUROC = 1.0) using both quantitation technologies. Time to results was greatly reduced using qPCR compared to NanoString (50 minutes vs. >18 hours).

Conclusions
Emergency department and hospital-based physicians currently rely on a battery of tests with moderate accuracy to diagnose acute infections and sepsis. HostDx Sepsis shows high accuracy for both diagnosis and prognosis of acute infections and sepsis in prospective studies. Any rapid, cartridge-based, sample-to-answer version of the HostDx Sepsis panel could allow for improved decision making for antibiotics, downstream testing, and level-of-care decisions.