1237 Multiplexing of an 18-Host-Gene Signature Using Rapid PCR for Better Antibiotics Decision

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Background

Physicians in outpatient settings and hospitals struggle with diagnosing acute infections and sepsis. This struggle has led to antimicrobial resistance, high mortality, and significant cost. Current diagnostic tests that require the pathogen to be present in blood are not sensitive enough. Recently, host response-based molecular diagnostics have emerged and are considered as a novel alternative or complimentary approach. From public and private microarray and next generation sequencing (NGS) data, we have previously developed, using bioinformatics tools, and validated an 18- gene signature set (11-gene sepsis panel and 8-gene fever panel) that can robustly distinguish between (i) noninfected, (ii) bacterial, and (iii) viral infection. Here we describe the translation of the 18-gene set into a rapid TaqMan multiplex qPCR assays. The signature set is being developed as a cartridge-based, sample-to-answer, quantitative assay with a turnaround time of less than 30 minutes.

Methods

To convert the biomarker set into rapid qPCR assays, 18 target genes were divided into groups of 4- or 5-plexes (RREB1 included as housekeeping control). Multiplex assays were verified and optimized in both singleplex and multiplex formats. Total RNA was extracted from blood samples collected in PAXgene® Blood RNA tubes from 21 patients (9 bacterial infections, 6 viral infections, 6 healthy controls), and used as templates for testing in parallel on the QuantStudio™ qPCR System and the NanoString nCounter® SPRINT, the latter being an amplification-independent gene quantitation platform.

Results

A correlation R value of 0.98 was obtained between the TaqMan PCR and NanoString platform across the 21 clinical samples tested. More importantly, based on calculated metascores from a subset of 11 genes, infected and noninfected individuals could be differentiated. Metascores from the other 8 genes differentiated bacterial from viral infected samples with high diagnostic accuracy.

Conclusion

An 18-gene signature set was successfully converted into multiplex TaqMan assays. As a rapid and accurate test, it promises to provide actionable results for improved antibiotics decision making for patients with suspected acute infections.