

An Ultra-Rapid Host Response Assay to Discriminate Between Bacterial and Viral Infections Using Quantitative Isothermal Gene Expression Analysis

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Background

Accurate diagnosis and treatment of bacterial infection is critical for improving patient outcomes. However, over-prescription of antibiotics has contributed to the emergence of antimicrobial resistant bacteria. Indeed, it is estimated that up to 20% of antibiotic prescriptions result in adverse effects¹. Discrimination between bacterial and viral infections currently relies on slow culture-based approaches or molecular pathogen detection which is inherently limited in scope. A rapid, point-of-care diagnostic capable of classifying infection types independent of pathogen identity would help to avoid unnecessary prescription of antibiotics to help curb the evolution of drug resistance and the incidence of ancillary infections. Here we report feasibility of such a test called HostDx™ Fever that is capable of separating infection types using an ultra-rapid isothermal amplification approach (Fig. 1).

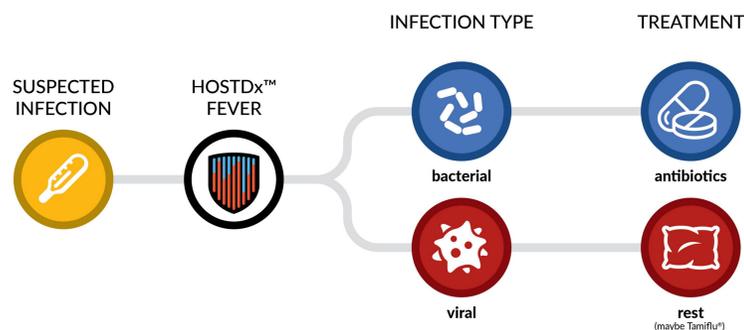


Figure 1. Diagrammatic workflow of antibiotic prescription decision making informed by HostDx™ Fever bacterial vs viral molecular diagnostic.

To identify host-response markers for this application, Sweeney *et al.* performed a multicohort analysis and derived a set of seven genes for robust discrimination of bacterial and viral infections². Performance for the presence of any bacterial infection in 20 pooled cohorts of 1,057 ED/ICU patients including children and adults from all continents showed a 94% sensitivity and 60% specificity (99% negative predictive value at 15% prevalence) resulting in a negative likelihood ratio (LR-) of 0.1². In a prospective clinical study of 96 pediatric ICU subjects LR- was upheld at 0.15³ (Fig. 2). To translate informatically identified markers to the benchtop, we next designed isothermal assays for rapid quantitation of mRNA expression from patient samples. Assays were developed for a subset of these markers to demonstrate feasibility of bacterial/viral discrimination by isothermal quantitation (Table 1, bold).

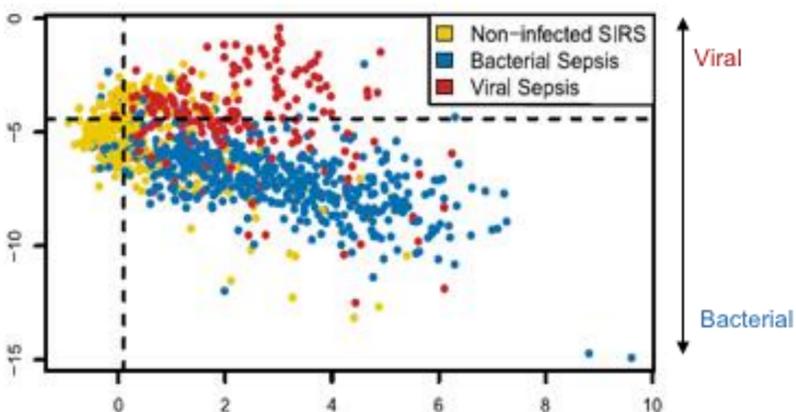


Figure 2. Distinction of infection from non-infection etiologies, and between bacterial and viral infections from Sweeney *et al.* Bacterial/viral score is plotted on the y-axis. Patients suffering viral infection (red) separate from those presenting bacterial infection (blue).

Results:

7 Genes in HostDx Fever Bacterial/Viral Panel Separated by Class

Bacterial	CTS ^B	GPA ^{A1}	HK ³	TNIP ¹
Viral	IFI²⁷	JUP	LAX ¹	

Table 1: Genes included in bacterial/viral panel separated by class. Those selected for an isothermal amplification feasibility study are shown in bold.

Assessing Linear Quantitative Range of Isothermal Assays by Serial Dilution

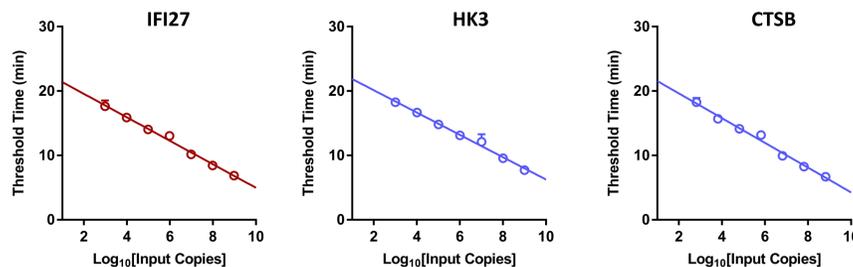


Figure 3. Threshold times for selected isothermal assays plotted as a function of log(input copy number) for serial dilutions of a control template. Results are fit by linear regression analysis; all R² = 0.99.

The specificity of each assay for mRNA was assessed by performing isothermal reactions using either human mRNA or genomic DNA as a template, with amplification observed only in reactions containing RNA (not shown). Serial dilutions of control material were evaluated for each assay to demonstrate a linear relationship between time to threshold (Tt) and the logarithm of input copy number. All assays have a linear dynamic range covering at least 6 orders of magnitude, indicating these assays can reliably quantitate target abundance within this range (Fig. 3). Linear regression analysis yielded standard curves by which target abundance could be calibrated between assays.

Correlation Analysis between Isothermal and nCounter® Marker Quantitation

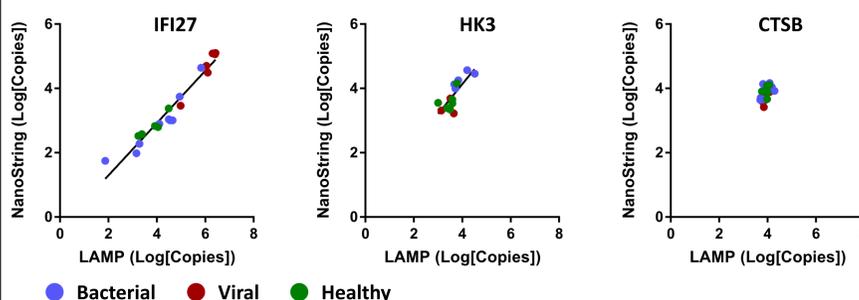


Figure 4. Correlation between isothermal and nCounter measurements of marker abundance in total RNA extracted from stabilized patient blood for selected bacterial/viral genes. Colors represent infection class: healthy: green, bacterial: blue, viral: red. Calculated Pearson correlation coefficients are inset.

Samples from healthy patients and patients presenting viral or bacterial infection were collected and processed to extract total RNA. Selected marker abundance in each RNA sample was measured by isothermal amplification and NanoString nCounter®. Mean time to result for isothermal analysis was 12.2 min compared to ~18 total hours for the nCounter. (Fig. 4).

Bacterial vs viral scores were calculated using the relative abundance data gathered by each method. Both methods demonstrate comparable separation of infection scores between bacterial and viral infection samples, indicating that isothermal amplification can reproduce sample infection scores obtained by amplification-independent measurement of marker mRNA (Fig. 5).

Separating Bacterial and Viral Infections using HostDx™ Fever Score

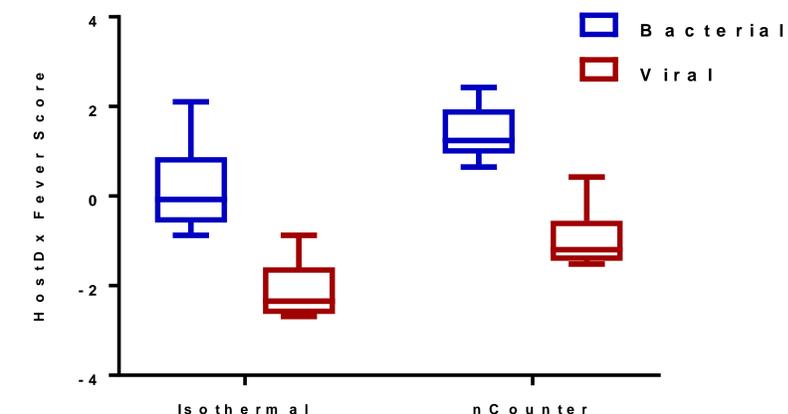


Figure 5. Comparison between HostDx Fever scores calculated based on mRNA abundance measurements made by isothermal or nCounter methods. Scores are separated by infection class; bacterial: blue, viral: red. Box: 25th-75th percentile, bars: sample range, line: sample median.

Conclusions

We have demonstrated:

- Wide linear dynamic range for isothermal assays
- Quantitative mRNA expression analysis by isothermal amplification
- Bacterial/viral infection discrimination by 3 HostDx Fever markers
- Time to result in less than 15 min

Methods

Sample Collection and Processing: Whole blood from healthy patients or patients suffering a bacterial or viral infection was collected in PAXgene™ Blood RNA tubes (PreAnalytix) and stored at -80°C prior to processing. Total RNA was extracted from these samples using a modified version of the accompanying PAXgene RNA blood kit protocol on a Qiacube® (Qiagen). RNA was quantified on a Qubit® fluorimeter (ThermoFisher).

Isothermal Amplification Assays: Standard LAMP assays consist of 6 primers, including forward/backward inner primers (FIP/BIP), forward/backward outer primers (F3/B3), and forward/backward rate enhancing primers (FR/BR)^{4,5}. FIP primers were designed to encompass a splice junction to specifically amplify mRNA. Assays were carried out using WarmStart LAMP 2X Master Mix (NEB) supplemented with fluorescent dye. Primers were added to final concentrations of 1.6 μM FIP/BIP, 0.2 μM F3/B3, and 0.4 μM FR/BR. Template was added in a standard 1 μL volume, and water was added to bring the final volume to 20 μL. Assays were distributed in 96-well plates for quantitative amplification on a QuantStudio® 6 Flex Real-Time PCR System (ThermoFisher).

NanoString Analysis: Amplification-independent mRNA abundance analysis was performed as the gold standard using the nCounter (NanoString) with fluorescent probes designed and manufactured by the vendor. In brief, target specific probes labeled with barcoded fluorescent tags are hybridized to target mRNA in a total RNA pool. Excess probes are removed and bound probes are counted by the instrument.

References

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