A Digital Microfluidic Sepsis Diagnostic Predicts Bacterial Infection Status Using Host Transcriptomic Response

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INTRODUCTION

- Early diagnosis of sepsis is critical due to its long-term health consequences and high mortality rate. However, typical diagnostic procedures for suspected sepsis include blood cultures that take hours to days to yield results.
- Differentiation between bacterial infection and illness due to other etiologies is necessary to inform intervention, including administering or withholding antibiotics, and prepare the warfighter to return to service.
- A rapid diagnostic test that predicts the likelihood of bacterial infection in suspected sepsis cases can help direct clinicians to appropriate treatment and may reduce unnecessary antibiotic use and help curb antimicrobial resistance.
- Here, we present a digital microfluidic (DMF) assay run on a point of care device and cartridge (Figure 1) that differentiates bacterial infection and non-bacterial illness using a panel of 10 mRNA targets.

METHODS

- A diagnostic assay was developed to differentiate bacterial infection from non-bacterial (viral or non-infectious) illness by interrogating the host immune response.
- The host response assay quantifies 10 host mRNAs along with two housekeeping controls used for normalization. RNA isolation and multiplex RT-qPCR are automated on the DMF cartridge (Figure 2).
- Machine learning techniques were employed to develop a logistic regression model that differentiates bacterial infection from non-bacterial illness using cycle threshold (C_t) values obtained on the DMF platform. Archived clinically adjudicated samples collected from 59 subjects admitted to the emergency room with suspected infection were run on the DMF host response assay, and the resulting data was used to train the predictive algorithm.
- Accuracy of the DMF host response assay was assessed by testing a separate set of 41 archived samples from subjects admitted to the emergency room with suspected infection.

DMF instrument *DMF cartridge*

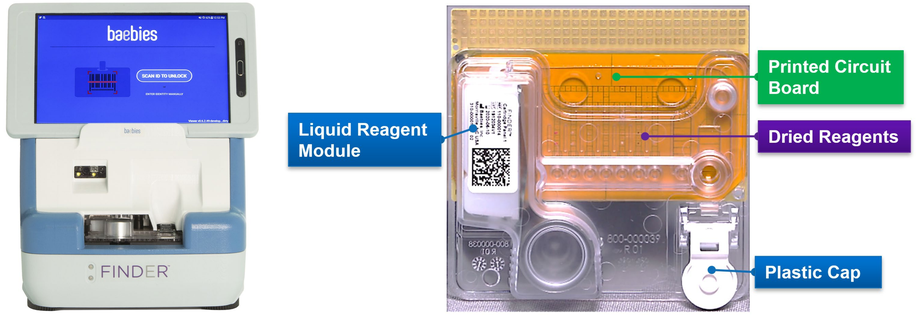

Figure 1. Baebies near-patient DMF platform for bacterial infection status determination.

Figure 2. DMF Host Response assay workflow.

RESULTS

- Consistent amplification of 12 host response mRNA targets can be achieved on the DMF cartridge, including two control targets used for normalization and 10 mRNA targets associated with infection status.

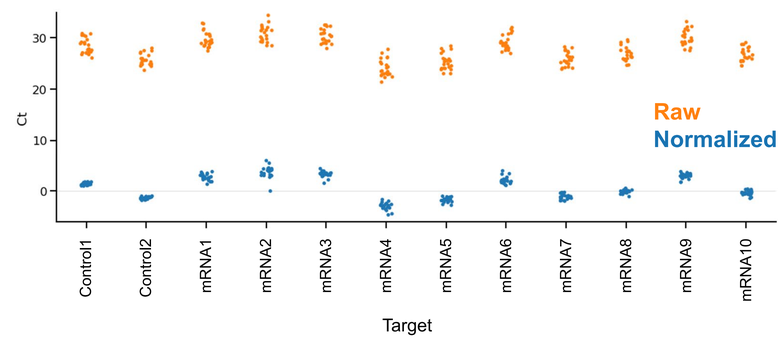

Figure 3. Raw (orange) and normalized (blue) C_t values of 12 mRNA targets obtained from 22 replicate runs of a single blood sample. Results demonstrate assay reproducibility across multiple cartridges.

- With a predictive algorithm trained using DMF data from 59 archived patient samples, the host response assay accurately predicted bacterial infection status of 41 additional samples with positive (PPA) and negative percent agreement (NPA) of 80% and 90%, respectively.

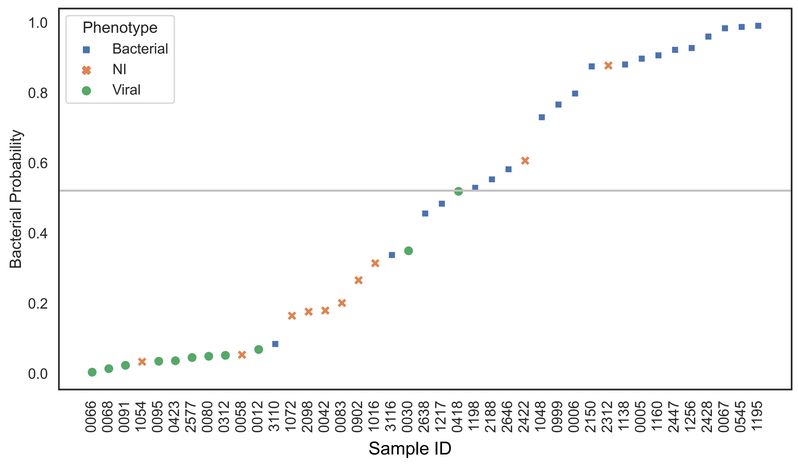

Figure 4. Probability of bacterial infection for 41 test samples as predicted by a model generated from data for 59 training samples. The horizontal line is the cut-off for classification into bacterial or non-bacterial categories. This cut-off was determined *a priori* during the training process. NI = Non-infectious illness.

Table 1. Bacterial vs. non-bacterial prediction by the DMF host response assay compared to phenotype determined by clinical adjudication, resulting in PPA=80%, NPA=90%. NI = Non-infectious illness.

		DMF Host Response Assay Prediction	
		Bacterial	Non-Bacterial (Viral or NI)
Clinical Adjudication	Bacterial	16	4
	Non-Bacterial (Viral or NI)	2	19

CONCLUSIONS

- We demonstrated feasibility of a point of care transcriptomic host response assay that discriminates bacterial infection from non-bacterial illness (80% PPA, 90% NPA) by analyzing 10 host mRNA markers from a single drop of blood (<50 µL).
- We envision that the DMF Host Response assay may serve as a complementary diagnostic test when assessing suspected sepsis cases, providing significant clinical information hours to days earlier than blood culture and reducing unnecessary antibiotic usage in the military.

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