Direct Detection of mRNA in Whole Blood Samples for Transcriptomic Profiling

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Introduction

Gene expression changes in circulating leucocytes reflect host response to many physiological and pathological conditions. The transcriptomic profile of leucocytes has been demonstrated to enable stratification of a patient population according to the etiology of diseases. A fast method to profile gene expression of host response to infection and inflammation can greatly impact the treatment decisions, course and outcome. The purpose of the present study was to demonstrate the ability of the Qvella™ FAST™ HR (Host Response) process to perform gene expression profile analysis on a whole blood sample in less than one hour.

Objective

The objective was to demonstrate proof of principle to measure host gene expression signatures in patient samples for viral and non-viral infection.

Method

Obtain samples in PAXgene™ tubes from human subjects with confirmed viral infection (n=5), bacterial infection (n=5), non-infectious illness (SIRS, n=5), and healthy controls (n=3)

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Perform Qvella FAST™ HR process to release stabilized mRNA in RT-PCR assay ready medium

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Detect Target and House-keeping Gene sets via Multiplexed Real-time RT-PCR

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Use cross-validated sparse logistic regression to quantify ability of normalized gene expression levels to discriminate viral samples in terms of classification accuracy

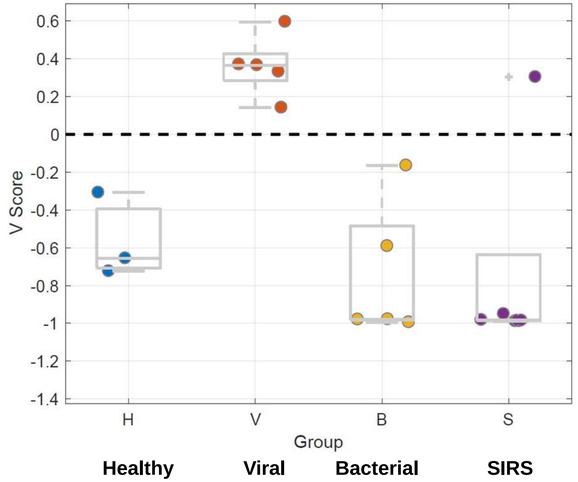
Repeatability of Qvella FAST™ HR Process

	Healthy Donor 1 (n=5)		Healthy Donor 2 (n=5)		Healthy Donor 3 (n=5)	
RT-PCR (Ct)	Average	STDEV	Average	STDEV	Average	STDEV
Infection Markers						
Marker 1	27.48	0.93	27.24	0.65	27.18	0.62
Marker 2	28.58	0.62	29.34	0.68	29.84	0.62
House-keeping Genes						
HPRT1	29.08	0.55	29.18	0.89	30.50	0.94
HSPCB	25.26	0.55	24.67	0.90	25.96	0.95

Results

The graph below plots the probability of viral Infection for whole blood samples processed and using Qvella FAST™ HR process, with target gene detection by RT-qPCR

Whole blood samples	3 healthy control
	5 viral infection
	5 bacterial infection
	5 SIRS (non-infectious)
Gene Targets	20 pan-viral infection markers
	2 housekeeping gene controls



A sparse logistic regression model using threshold cycle values resulted in 94% (17/18) leave-one-out cross-validated accuracy. The only misclassification was a subject with SIRS (non-infectious) classified as viral.

The reproducibility of the measured relative threshold cycle values was <1 cycle based on the standard deviations over different sample preparations across multiple samples from different healthy donors.

Conclusion

This feasibility study successfully measured host gene expression signatures in about 45 minutes in a sample-to-answer format to discriminate viral from non-viral infection.