Panomics

Biomarker Analysis

Quantitative Gene Expression Directly from Dried Blood Spots

Introduction

Archival blood collected on Guthrie card filter paper, commonly called "Guthrie cards", serve as a valuable resource for retrospective genetic studies. The ease of storage and transportation of these cards enables a variety of large field studies. It has been reported that proteins and DNA may be recovered from these cards after extended periods of storage. RNA has been considered too vulnerable to degradation by ribonucleases to be recovered from these filters; however, recent reports showed that undegraded mRNA could be obtained from dried bloodstains on filter paper after up to 27 years of storage[1].

Current Challenges

Although dried blood spots (DBSs) offer an easy and inexpensive means for the collection and storage of blood, the amount of blood sample spotted on filter paper is limited. The efficient recovery of RNA from dried blood may be problematic, because currently available methods for analyzing RNA from dried blood spots requires RNA isolation[1,2]. In addition to the RNA extraction step, the subsequent enzymatic reactions such as reverse transcription and Polymerase Chain Reaction make the precise and accurate quantification of RNA in dried blood samples even more difficult.

QuantiGene® Branched DNA Solution

Branched DNA technology (bDNA) is a sandwich nucleic acid hybridization assay that is the basis for Bayer's FDA approved clinical tests for HIV and hepatitis. The technology has been licensed and further developed by Panomics, and is offered as a single gene (QuantiGene) or up to 30-gene multiplexed assay (QuantiGene Plex). QuantiGene and QuantiGene Plex Blood assays measure RNA directly from a dried blood spot lysate, without RNA isolation or target amplification, providing an invaluable tool for analysis of DBS specimens that are limited in quantity and non-replenishable due to their archival nature.

Highlights

No RNA purification
Quantitate mRNA directly from dried blood spot lysates

No Reverse Transcription
Eliminate biases against messages that do not transcribe well

No Target Amplification
Eliminate biases again messages that do not amplify proportionately due to either random events or sequence composition

Overcome Sample Limitation and RNA Quantity Issues
Direct RNA quantitation without RNA purification or target amplification

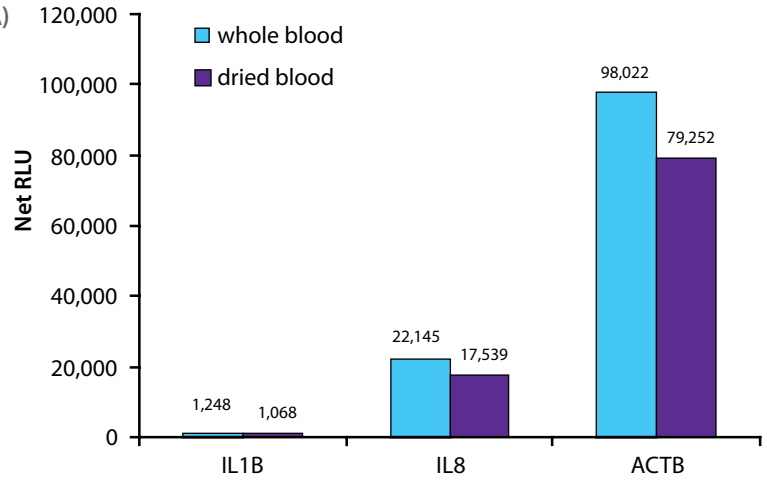
Precise and Accurate
Minimize sample handling and simplify workflow

Flexible Format
Single target or multiplex (3-30) assays

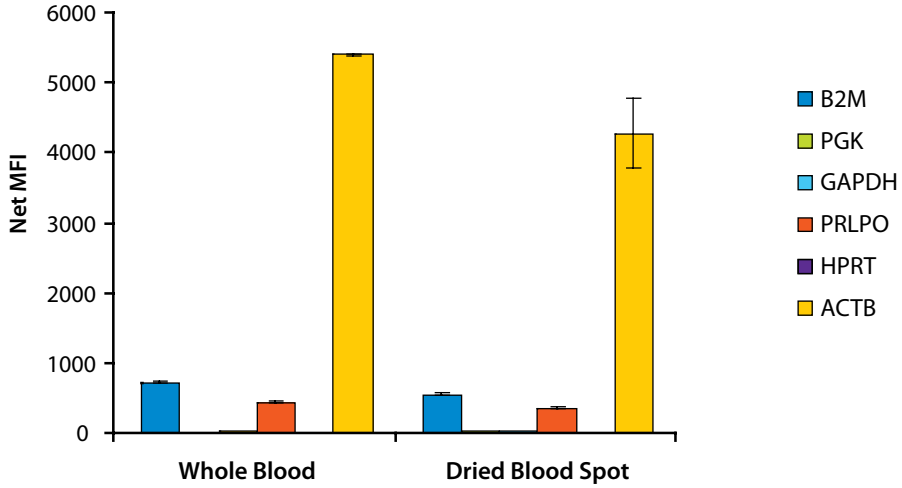
Efficient mRNA Recovery from Dried Blood Spots

Gene expression measurements were obtained from a dried blood spot and the equivalent amount of whole blood from the same individual. The signals for the dried blood spot samples were 80% of the control whole blood sample. (A) A QuantiGene Blood assay measured beta actin, IL8 and IL1B (representing high, medium and low abundance genes). (B) A QuantiGene Plex Blood assay measured a panel of six housekeeping genes with varying expression levels. (C) Signals from the QuantiGene Plex Blood assay with 3 sample inputs from dried blood spot lysates and the equivalent 2.5, 5 and 10 µL of whole blood show excellent correlation, R^2>0.99.

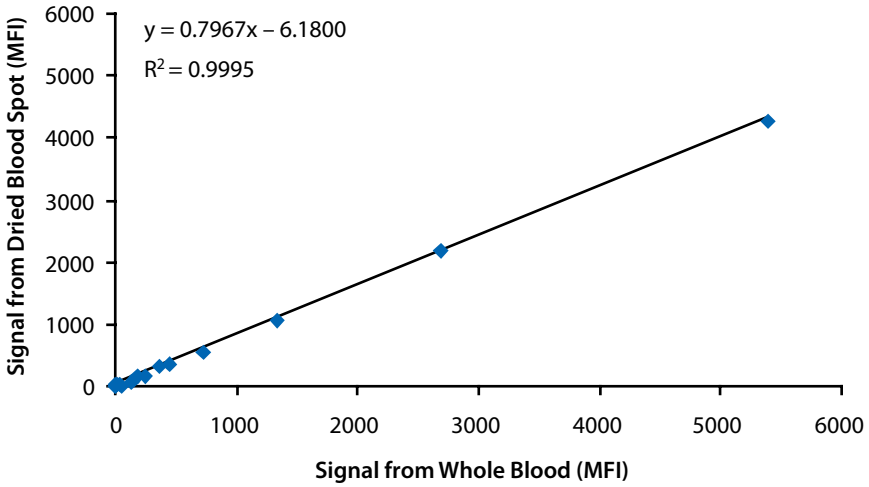
A)



B)

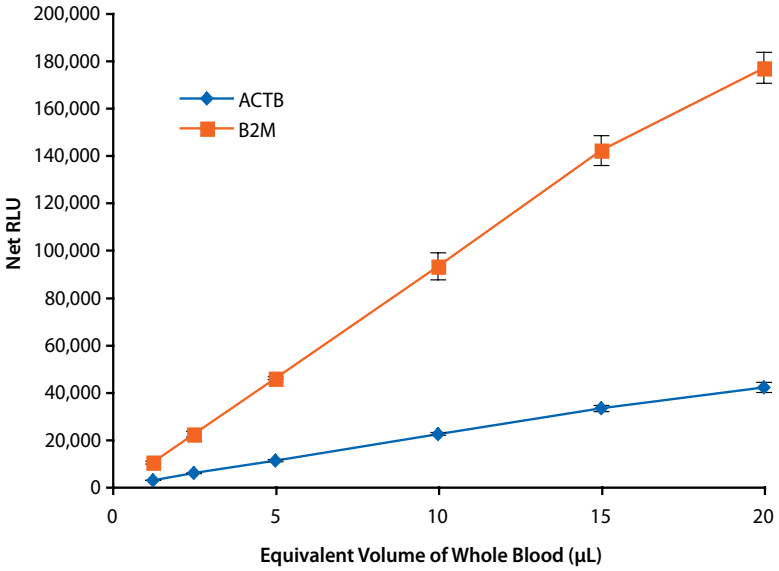


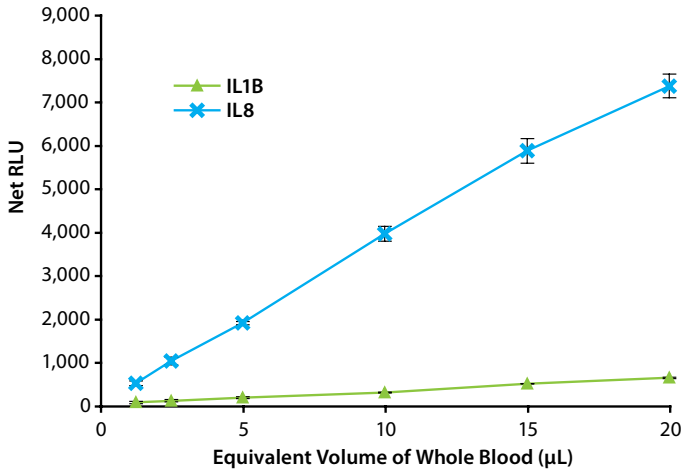
C)



Assay Performance—Sensitivity and Linearity

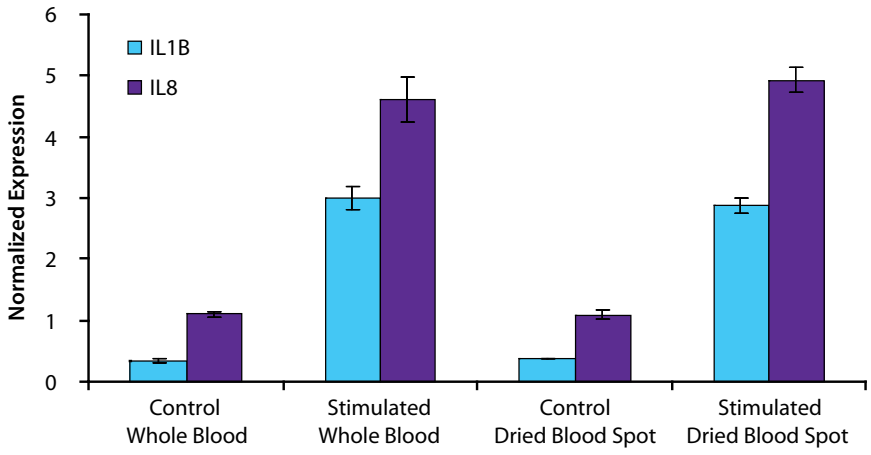
A dilution series, prepared from a dried blood spot lysate, contained an equivalent of 1.25–20 µL of whole blood. Four targets, ranging from low to high abundance, were evaluated to access sensitivity and linearity. All four genes showed excellent linearity and sensitivity, R^2 values of > 0.99 and reliable signals for 1.25 uL equivalent of whole blood. Assay coefficients of variation (CV%) for the 28 data points (n=3 for each sample) ranged from 0–10% with an average CV of 4.2%.





Equivalent Cytokine Induction from Dried Blood Spots and Whole Blood

After treatment with LPS or vehicle for 60 minutes, half of the whole blood was spotted on filter paper, and the other half was lysed immediately for the QuantiGene Blood assay. Target gene expression level was normalized to beta actin. Expression levels of IL1B and IL8 were significantly induced by LPS, consistent with a previous report[3].



References

1. Guthenberg, C. et al. 2003. Extraction of RNA from dried blood on filter papers after long-term storage. Clinical Chemistry 49(6): 979-981.
2. Suttorp, M. et al. 1997. Blood on filter paper as a readily available source of bcr-abl rearranged mRNA. Blood 90(4): 1713-1715.
3. De Groote D. et al. 1992. Direct stimulation of cytokines (IL-1 beta, TNF-alpha, IL-6, IL-2, IFN-gamma and GM-CSF) in whole blood. I. Comparison with isolated PBMC stimulation. Cytokine 4:239–248.

Ordering Information

QuantiGene Blood and QuantiGene Plex Blood Reagent Systems are both comprised of 3 modules. Each of the modules is sold separately and is available in multiple sizes.

The QuantiGene and QuantiGene Plex Blood Sample Processing Kit for Whole Blood is required for working with dried blood spots.

QuantiGene Blood Reagent System

Product	Size	Cat. No.
QuantiGene Blood Assay Kits		
QuantiGene Blood Assay Kit	2-Plate	QB0001
QuantiGene Blood Assay Kit	10-Plate	QB0002
QuantiGene Blood Assay Kit	5 x 10-Plate	QB0003
QuantiGene Blood Sample Processing Kits		
QuantiGene Blood Sample Processing Kit—Whole Blood	2-Plate	QB0100
	10-Plate	QB0101
QuantiGene Blood Sample Processing Kit—PAXgene Blood	192 samples*, 2-Plate	QB0102
	960 samples*, 10-Plate	QB0103
** Each prepared PAXgene blood sample is sufficient for running 4 assay wells.*		
QuantiGene Blood Target-Specific Probe Sets		
QuantiGene Blood Probe Set, Catalog	200 Rxns	See Website
	1,000 Rxns	See Website
QuantiGene Blood Probe Set, By Request	200 Rxns	QB0050
	1,000 Rxns	QB0051
QuantiGene Blood Products for New Users		
QuantiGene Blood Evaluation Kit—Whole Blood	2-Plate	QB0004
QuantiGene Blood Evaluation Kit—PAXgene Blood	2-Plate	QB0005

QuantiGene Plex Blood Reagent System

Product	Size	Cat. No.
QuantiGene Plex Blood Assay Kits		
QuantiGene Plex Blood Assay Kit	1-Plate	PB0001
QuantiGene Plex Blood Assay Kit	3-Plate	PB0002
QuantiGene Plex Blood Assay Kit	10-Plate	PB0003
QuantiGene Plex Blood Sample Processing Kits		
QuantiGene Plex Blood Sample Processing Kit—Whole Blood	1-Plate	PB0100
	3-Plate	PB0101
	10-Plate	PB0102
QuantiGene Plex Blood Sample Processing Kit—PAXgene Blood	96 samples*, 1-Plate	PB0103
	288 samples*, 3-Plate	PB0104
	960 samples*, 10-Plate	PB0105
** Each prepared PAXgene blood sample is sufficient for running 4 assay wells.*		
QuantiGene Plex Blood, Plex Sets		
QuantiGene Plex Blood, Plex Set, By Request	96 Assays, 03–30 Plex	800000-1##
	3 x 96 Assays, 03–30 Plex	800000-2##
	10 x 96 Assays, 03–30 Plex	800000-3##
QuantiGene Plex Blood, Plex Set, Catalog	Multiple	See Website
## Defines the plex number from 03–30.		

For pricing and more information visit our website at www.panomics.com or call us at 1.877.726.6642.

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