Stabilization of Gene Expression Profiles in Post-Phlebotomy Whole Blood Using the *PAXgene™ Blood RNA System

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ABSTRACT

A major impediment to accurate analysis of gene expression in whole blood is the post-phlebotomy change in cellular transcript patterns. Nuclease degradation of RNA as well as non-specific gene induction is triggered minutes after blood collection and continues during sample transport and processing.

We have developed an evacuated blood collection tube containing a stabilizing additive and a chemically linked companion sample processing system (PAXgene) that: 1) stabilizes cellular RNA in whole blood and, 2) purifies, via spin-column technology, high quality total RNA. We compared gene expression profiles of room temperature stored PAXgene and unpreserved (EDTA) whole blood using quantitative PCR (ABI Prism 7700 TaqMan™) assays for 36 gene transcripts (Source Precision Profile™ for Inflammation) optimized for precision (repeatability: C.V. <2%). RNA was purified from PAXgene and unpreserved EDTA whole blood at the time of phlebotomy (t_0) and 4, 8, 24, 72 and 120 hours post-phlebotomy.

Eleven of 36 transcripts were undetectable in either PAXgene or EDTA blood. mRNA expression levels were unchanged in 3 of 25 measurable transcripts in both donors in EDTA blood; the relative amount of each of the remaining 22 transcripts changed by up to 10^4-fold. By contrast, 23 of 25 measurable transcripts were stable in PAXgene blood in one or both donors for 5 days at ambient temperature. The %CV of the PAXgene Blood RNA System for three donors (8 tubes/donor) were 7-24% for total RNA yield and 1% for C_T values in a GAPDH RT PCR assay. The ability to stabilize and purify high quality RNA using the PAXgene Blood RNA System, coupled with Source Precision Medicine's optimized quantitative PCR, enables routine use of gene expression analysis in the evaluation of clinical samples.

*The PAXgene™ Blood RNA System is for research use only. Not for use in diagnostic procedures.

INTRODUCTION

- Accurate analysis of gene expression in whole blood is negatively affected by post phlebotomy changes in cellular transcript patterns caused by:
  - Rapid *ex vivo* degradation of cellular RNA by endogenous nucleases
  - Gratuitous gene induction precipitated by phlebotomy and sample handling.
- We have developed the PAXgene™ Blood RNA System consisting of:
  1) a direct-draw, evacuated blood collection tube containing a stabilizing additive
  2) a companion sample processing system for the isolation of high quality intracellular total RNA.
- Here we discuss the use of the PAXgene™ Blood RNA System for stabilizing gene transcription profiles and total cellular RNA in whole blood stored at at 22°C, 4°C and -20°C.

METHODS

Sample Collection and Storage

- Patient population consisted of consenting normal adult donors.
- Using a BD Vacutainer™ Blood Collection Set, whole blood was collected by phlebotomy from each donor into Vacutainer™ PLUS K_2EDTA (EDTA) and PAXgene™ Blood RNA Tubes (PAXgene).
- Blood samples were stored *in situ* for the designated times at designated temperatures prior to RNA extraction.

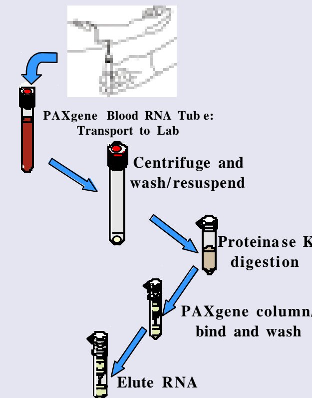
RT PCR

- Reverse transcription synthesis was performed on 1.5 to 3 µg of total RNA
- Primed with random hexamers using Multi Scribe™ (Applied Biosystems, AB) reverse transcriptase using the manufacturer's protocol
- 18S rRNA used as internal standard for quantifying transcripts. (18S rRNA amplifying at 12-14 C_T, $\Delta\Delta$ C_T Method).
- qPCR determined using Source Precision Profiles™ on an AB 7700 Sequence Detection System

RNA Isolation from EDTA Tubes

- One ml of blood is added to a centrifuge tube containing 6 mls Trizol™ (Life Technologies) reagent + 1 ml RNAse-free water and mixed well.
- Add chloroform, mix, and centrifuge to perform organic extraction.
- Remove aqueous phase containing nucleic acids and perform isopropyl alcohol precipitation of RNA
- Wash the nucleic acid pellet with 75% ethanol.
- Trizol isolated RNA is applied to a QIAGEN RNeasy® spin column and washed.
- Contaminating DNA is digested using QIAGEN RNase-free DNase Set on-column procedure.
- RNA is eluted with RNase-free water.

PAXgene Blood RNA Isolation



RESULTS

Total RNA Recovery from PAXgene and EDTA Blood Stored at 22°C

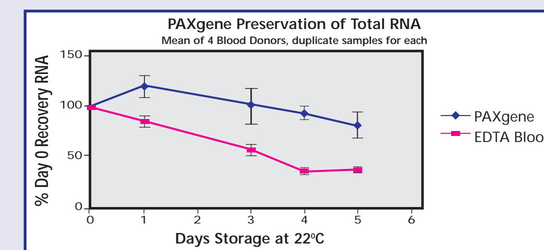


Figure 1: Storage and recovery of total RNA from whole blood stored in Vacutainer™ PLUS K_2EDTA and PAXgene™ Blood RNA Tubes for 5 days at 22°C. Averages of four donors, blood processed in triplicate, error equals standard error of the mean.

Total RNA Recovery from PAXgene and EDTA Blood Stored at 4°C and -20°C

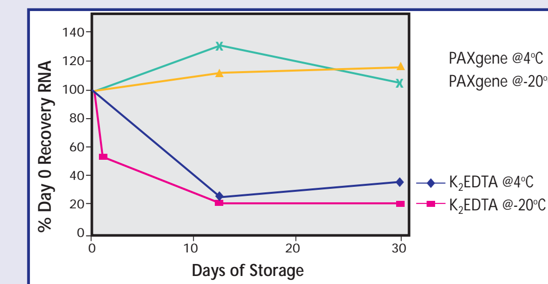


Figure 2: Long term storage and recovery of total RNA from whole blood stored in Vacutainer™ PLUS K_2EDTA and PAXgene™ Blood RNA Tubes for 30 days at 4°C and –20°C. Single donor, blood processed in triplicate.

Reproducibility of PAXgene Blood RNA System

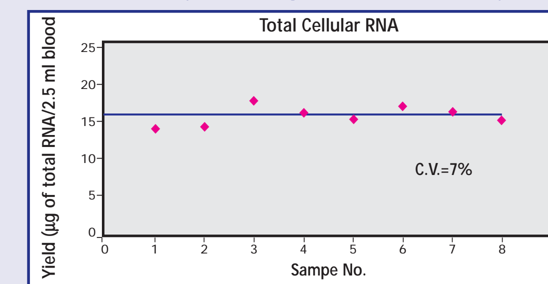


Figure 3: Reproducibility of PAXgene™ Blood RNA System. Eight PAXgene RNA Tubes collected per donor and stored at room temperature for 24 hours prior to RNA extraction. Total RNA purified using PAXgene™ Blood RNA Kit protocol and reagents. Results shown for single donor.

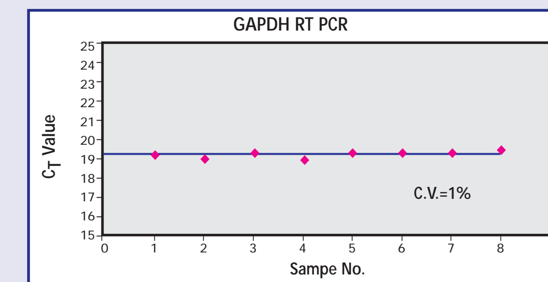
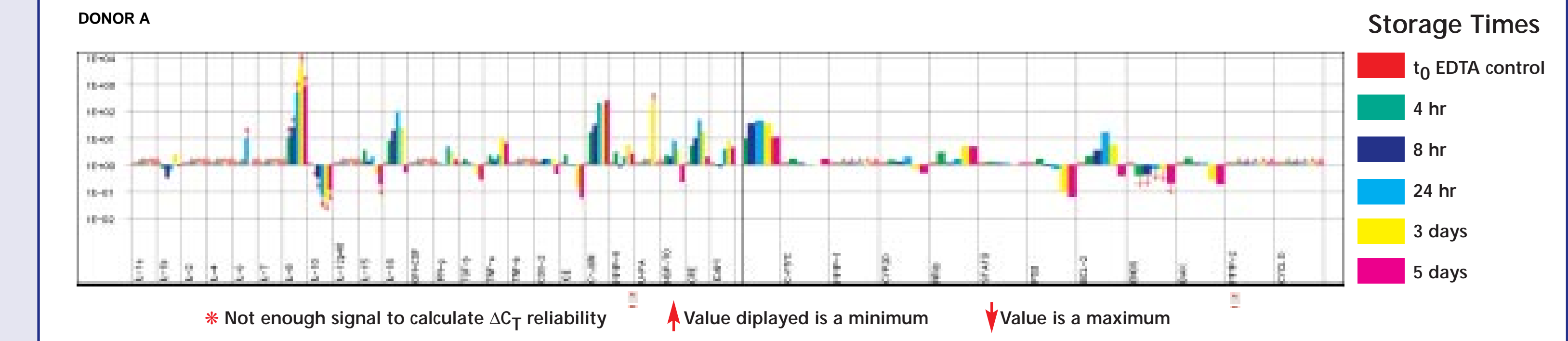


Figure 4: GAPDH RT PCR results of RNA preparations shown in Figure 3 above. 2µl of spin column eluate analyzed in a real-time PCR (TaqMan™) GAPDH assay.

Stability of Transcription Profile: Real Time PCR †Source Precision Profile™

EDTA Tubes: Storage at 22° C for five days



PAXgene™ Tubes: Storage at 22° C for five days

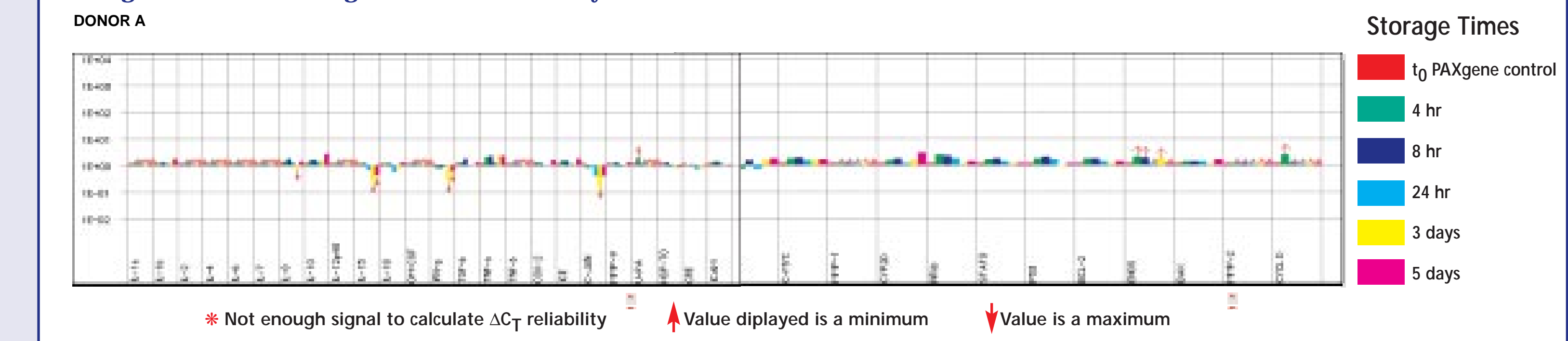


Figure 5: Example of a 36-gene transcription profile of single donor whole blood collected in EDTA Tubes (top panel) or PAXgene™ Blood RNA tubes (bottom panel). Samples were stored at 22°C for 10minutes (EDTA tube t_0 control), 2 hours (PAXgene t_0 control) and 4 hours, 8 hours, 24 hours, 3 days and 5 days (EDTA and PAXgene test samples). Total RNA extracted from EDTA tubes using Trizol method. Total RNA extracted from PAXgene™ Tubes using PAXgene™ Blood RNA System Kit. Graph shows mRNA copy number normalized to an 18S endogenous control and relative to tube t_0 control copy number.

SUMMARY

- **In comparison to EDTA tubes, total cellular RNA in whole blood is stabilized in PAXgene Blood RNA Tubes for up to 5 days at 22°C and for a minimum of 30 days at 4°C and -20°C.**
- **Over the time period studied, 23 of 25 measurable transcripts were stabilized in PAXgene whereas the relative copy number for these transcripts changed by up to 10^4-fold in EDTA tubes.**
- **The PAXgene Blood RNA System appeared to inhibit or eliminate gratuitous gene induction in whole blood caused by phlebotomy and sample storage procedures.**
- **The PAXgene Blood RNA System is highly reproducible as measured by %CV's results from multiple samples from the same donor: 7-24% for total RNA yield and 1% for C_T values in a GAPDH RT PCR assay.**

†Source Precision Profiles™ are calibrated measures of bio-responses based on quantitative gene expression. The Source Precision Profile platform is based on a quantitative PCR (ABI Prism 7700 TaqMan™) system optimized for precision (repeatability; coefficient of variation < 2%) and backed by strict SOPs, providing quantitative and reproducible data.