

Stabilization of cellular RNA in whole blood samples using the PAXgene™ Blood RNA System

L. Rainen, C. Ballas, U. Oelmueller*, S. Jurgensen†, R. Wyrich*, J. Schram†, M. Walenciak, C. Herdman†, M. Paumen*

PreAnalytix (CH), c/o BD, Franklin Lakes, NJ, USA.

* PreAnalytix (CH), c/o QIAGEN GmbH, Hilden, Germany

† BD Technologies, RTP, NC, USA

A major difficulty in studying gene expression patterns in whole blood is that extensive changes in transcript levels occur during phlebotomy and during subsequent storage or transport of samples. In order to address this problem, PreAnalytix developed the PAXgene™ Blood RNA System, an integrated and standardized system for the collection and stabilization of whole blood samples and isolation of cellular RNA. The system consists of PAXgene Blood RNA Tubes and the PAXgene Blood RNA Kit. PAXgene Blood RNA Tubes are used for blood collection, and contain a proprietary blend of reagents that provides immediate stabilization of RNA, allowing blood samples to be stored for days at 18–22°C (exact period depends on the downstream application). This blend also prevents the drastic changes in cellular RNA expression profiles that normally take place after blood collection. RNA is then isolated using proven silica-gel-membrane technology supplied in the PAXgene Blood RNA Kit.

Safe Storage for 30 Days Using the PAXgene System

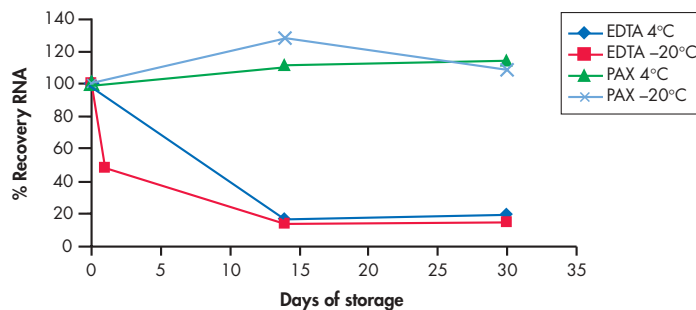


Figure 2 Blood was collected in PAXgene Blood RNA Tubes and RNA isolated using the PAXgene Blood RNA Kit, or collected in EDTA tubes and RNA isolated without stabilization, as described in “Materials and methods”. Samples were stored for up to 30 days at 4°C or -20°C. Samples were taken from one donor and processed in triplicate (mean values are shown).

We compared RNA yields obtained using the PAXgene Blood RNA System with yields obtained using a method that did not include an RNA stabilization step. The performance of RNA isolated using the PAXgene System in downstream PCR was assessed.

Materials and methods

Blood samples were taken from adults with informed consent. Blood was collected either in BD Vacutainer™ PLUS K₂EDTA Tubes, or in PAXgene Blood RNA Tubes, and stored at -20°C, 4°C, and room temperature for up to 30 days. RNA was isolated from samples collected in EDTA tubes using a commercial ▶

High Yields of RNA Using the PAXgene System

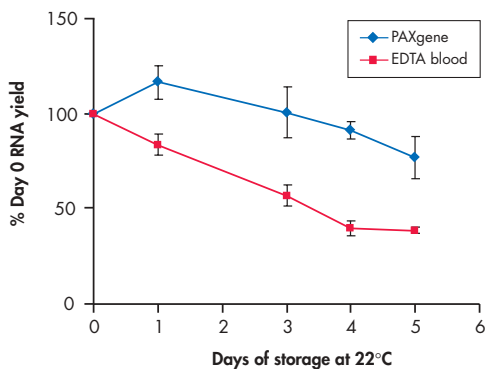


Figure 1 Blood was collected in PAXgene Blood RNA Tubes and RNA isolated using the PAXgene Blood RNA Kit, or collected in EDTA tubes and RNA isolated without stabilization, as described in “Materials and methods”. Samples were stored for up to 5 days at room temperature. Samples were taken from 4 blood donors and processed in triplicate (mean values are shown)

The PAXgene Blood RNA System Provides Highly Reproducible Results

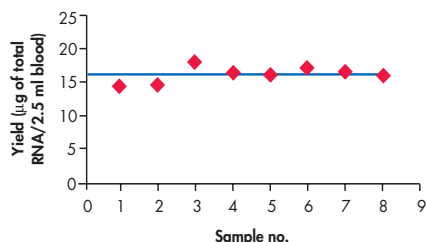


Figure 3 Eight blood samples were taken from each donor and stored at room temperature for 24 hours before RNA purification. Yields are shown for a single donor.

phenol-based reagent (Supplier I) according to the manufacturer's instructions, followed by cleanup using a silica-based method (no RNA stabilization step). Residual DNA was removed using an optional on-column DNase treatment, and RNA was eluted in RNase-free water. RNA was isolated from samples collected in PAXgene Blood RNA Tubes using the PAXgene Blood RNA Kit, including the optional on-column DNase treatment. Quantitative, real-time RT-PCR was performed using the TaqMan® system and primers designed to amplify the GAPDH gene, using 2 µl of eluate from the PAXgene System as a template.

Results and discussion

RNA yields were higher during 5 days of blood storage at 18–22°C when using the PAXgene System than when using a collection system that did not include an RNA stabilization step (Figure 1). The loss in RNA yield from blood stored in EDTA tubes indicates RNA degradation. When blood was stored at 4°C or –20°C, the PAXgene System provided constant RNA yields for a minimum of 30 days. In contrast, RNA yields decreased significantly when blood was collected and stored in conventional EDTA tubes (Figure 2). The PAXgene System yielded highly reproducible results (Figure 3), and the resulting RNA performed well in quantitative, real-time RT-PCR (Figure 4).

Conclusions

Use of the PAXgene Blood RNA System provides efficient stabilization of cellular RNA in whole blood during and after phlebotomy. Excellent and reproducible yields of RNA are achieved. ■

Excellent Results in Quantitative, Real-Time RT-PCR

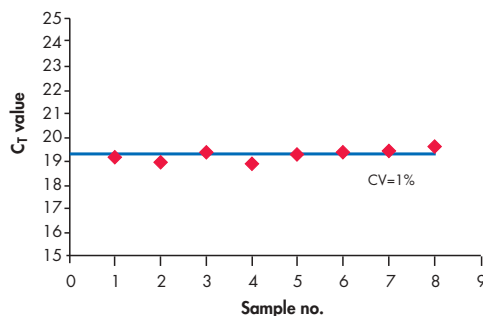


Figure 4 The RNA samples analyzed in Figure 3 were amplified in quantitative, real-time RT-PCR using the TaqMan system with 2 µl of each eluate.

Ordering Information

Product	Contents	Cat. No.
PAXgene Blood RNA Tubes (100)*	100 blood collection tubes. To be used in conjunction with the PAXgene Blood RNA Kit (50).	762115,† 762125‡
PAXgene Blood RNA Kit (50)*	50 PAXgene Spin Columns, Processing Tubes, RNase-free Reagents and Buffers. To be used in conjunction with PAXgene Blood RNA Tubes.	762134
PAXgene Blood RNA Validation Kit (10)*	10 blood collection tubes, 10 PAXgene Spin Columns, Processing Tubes, RNase-free Reagents and Buffers	762132

* For research use only. Not for use in diagnostic procedures.

† USA and Canada

‡ All other countries