

Stabilization of gene expression in whole blood: high quality RNA with room temperature handling of human blood samples.

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Abstract

Gene expression profiles from blood samples are being increasingly used to diagnose and monitor disease progression and assess patient responses to treatment. This requires reliable preservation of RNA in samples during collection, transport and storage. The highly dynamic transcription profiles can change rapidly after blood collection, potentially affecting interpretation of gene expression, and ultimately dictate inadequate treatment. Biomatrix has developed a collection device for whole blood, the RNAgard® Blood Tube, which stabilizes the RNA in blood cells and allows for ambient temperature sample handling and storage. We have also developed a robust RNA purification method which, when coupled with RNAgard Blood Tubes, provides a complete solution for blood collection, storage and RNA purification. We present data validating higher yields and greater quality of RNA than the leading competitor's blood collection tubes, while maintaining a gene expression profile similar to that obtained at the time of collection. Comparative testing shows that blood in Biomatrix's RNAgard Blood Tubes retain high quality RNA for much longer storage periods, and exhibit significantly less transcriptome variability than the leading competitor. Biomatrix's RNA preservation and purification solution can serve as a reliable entry point for research and molecular diagnostics applications. It is more effective in delivering accurate gene expression patterns than alternative blood RNA collection devices, and it should prove of extreme value for better patient treatment.

Materials and Methods

Sample preparation and nucleic acid analysis:

Human whole blood was collected in RNAgard Blood (RGB) Tubes or competitor RNA preservation tubes and stored as stated in each figure. RNA isolation was performed using the BioMaxi™ Blood RNA Purification Kit, QIAcube® System, or the competitor RNA isolation kit. Integrity of RNA was assessed by agarose gel electrophoresis, Agilent 2100 Bioanalyzer and RNA 6000 Nano Kit, or by RT-qPCR and Illumina Human HT-12 gene expression array.

Gene expression analysis:

Purified RNA samples were reverse transcribed, and specific cDNAs amplified using the iScript Reverse Transcription Supermix (BioRad). Differences in IL1-β and c-Fos expression were calculated relative to expression at time "0" using the ΔΔCt method. RNA integrity and quality were assessed by agarose gel electrophoresis and Agilent 2100 Bioanalyzer. RNA samples were then analyzed with Illumina HT-12 gene expression array according to the manufacturer's instructions.

Results

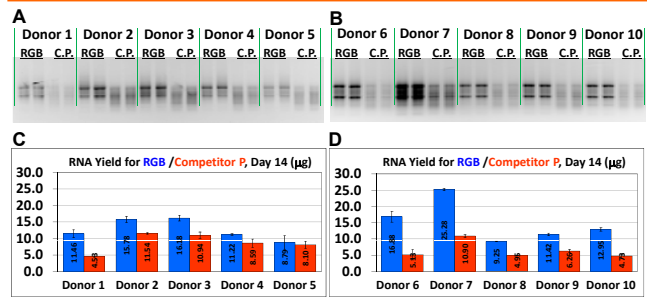


Figure 1. RNAgard Blood Tubes stabilize blood samples at room temperature for at least 14 days. Blood from 10 healthy human donors was collected in RNAgard Blood or competitor's blood tubes (Competitor P; C.P) and stored at room temperature for 14 days. RNA was purified using Biomatrix's BioMaxi Blood RNA Purification Kit or the competitor's recommended kit. The RNA samples were analyzed by agarose gel electrophoresis (A,B). Total RNA yield per blood tube was determined by UV spectroscopy, (C,D).

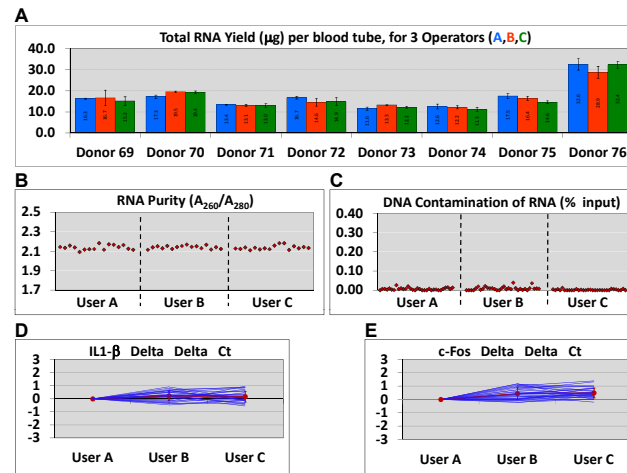


Figure 2. RNA with high quality and purity from blood samples collected in RGB Tubes using the BioMaxi RNA Purification Kit. Blood from 8 healthy human donors was collected in RGB Tubes and stored at room temperature for 3 days. RNA was isolated by 3 different operators (A,B,C). The total RNA yield per blood tube and RNA purity (A_{260}/A_{280}) were determined by UV spectroscopy (A,B). Percent of genomic DNA contamination in RNA samples was determined against a standard curve by qPCR amplification of an RNase P amplicon (C). Expression of IL1-β (D) and c-Fos (E) transcripts for all samples were determined by quantitative RT-PCR, relative to the expression values obtained by User A, using 18S rRNA as an internal control.

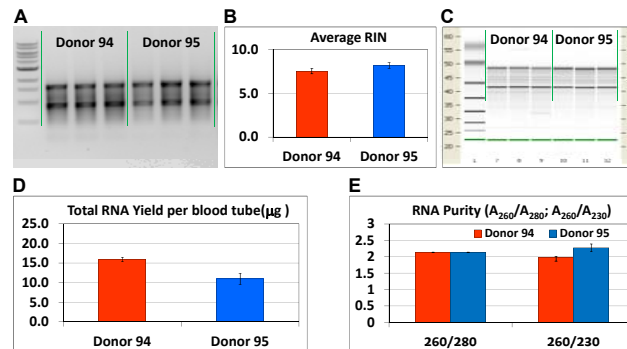


Figure 3: Automation of RNA isolation from blood samples collected in RNAgard Blood Tubes. Blood from two donors was drawn into RNAgard Blood Tubes and stored for 14 days at room temperature plus an additional 2 days at 4°C. RNA isolations were performed using an automated method (QIAcube). Samples were analyzed by agarose gel electrophoresis (A). RNA Integrity Numbers (RIN) were determined (B), and the average RIN for each donor was calculated (C). Total RNA yield per blood tube (D) and RNA purity (A_{260}/A_{280}), (E) was determined by UV spectrophotometry.

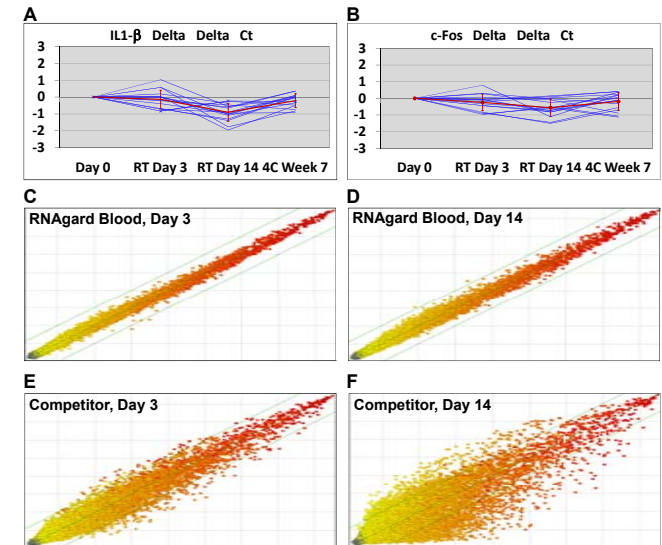


Figure 4. Highly stabilized gene expression in blood stored in RNAgard Blood Tubes for up to 2 weeks. A,B: After the indicated sample storage, RNA was purified from duplicate blood samples stabilized in RNAgard Blood Tubes. Levels of IL1-β (A) and c-Fos (B) transcripts were determined relative to the values obtained for Day 0 by real-time RT-PCR using 18S rRNA as control. (C-F) Gene expression profile of ~34,000 genes using the Illumina Human HT12 Bead Array, for RNA isolated from blood collected in RNAgard Blood Tubes or competitor tubes after 3 days (C,E) or 14 days (D,F) of room temperature storage, relative to RNA isolated from freshly collected blood. Plots outside the green lines correspond to > 2-fold change in expression levels relative to samples processed on the day of blood collection.

Conclusions

- RNAgard Blood Tubes protect RNA in blood samples stored at ambient temperature for at least 14 days, clearly outperforming the leading competitor's blood collection tubes (Fig. 1).

- High yield of RNA with excellent quality and purity can be reproducibly isolated from blood samples collected and stored in RNAgard Blood Tubes, using Biomatrix's BioMaxi Blood RNA Purification Kit (Fig. 2).

- RNA isolation from blood samples collected and stored in RNAgard Blood Tubes can be easily automated using Qiagen's QIAcube sample prep platform, resulting in high yields of RNA with great purity and integrity (Fig. 3)

- Gene expression remains unaltered in blood samples stored in RNAgard Blood Tubes for at least 14 days at room temperature and 7 weeks at 4°C (Fig. 4 A,B)

- The total gene expression profile of blood stored for 3 or 14 days at room temperature in Biomatrix's RNAgard Blood Tubes remain unaltered, comparable to that of freshly collected blood samples, clearly outperforming the leading competitor (Fig. 4 D-F)