PreAnalytiX
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Technical Note
PAXgene® Blood RNA System

In situ stability of RNA in blood samples stored for 5 years (60 months) at –20°C and –70°C in PAXgene Blood RNA Tubes

Study Design

For each study, blood was drawn into PAXgene Blood RNA Tubes from a minimum of 10 consenting adult donors with white blood cell (WBC) counts in the normal range of 4.8 – 11.0 x 10^6 WBC/ml blood. Replicate specimens were stored in situ at either –20°C or –70°C and processed in duplicate at the indicated time points* in accordance with the *PAXgene Blood RNA Kit Handbook* (manual protocol).

The integrity of purified RNA was analyzed by capillary gel electrophoresis using RNA 6000 Nano reagents and chips on an Agilent® Bioanalyzer 2100† (Agilent Technologies). Moreover, the RNA was tested in quantitative RT-PCR assays for FOS and IL1B.

Note: The real-time duplex RT-PCR assays used in this study required use of an ABI PRISM® 7900HT Sequence Detection System instead of the ABI PRISM 7700 Sequence Detection System previously used in stability studies with shorter blood storage times.

* This study is continuing for 10 years.

† RNA integrity results provided for supporting data only; no claims for RNA integrity are made for the PAXgene Blood RNA System.

Results

Stability of RNA in blood stored in situ at –20°C

Figures 1 and 2 show the change in relative FOS and IL1B transcript levels for RNA in blood stored in situ in PAXgene Blood RNA Tubes at –20°C.

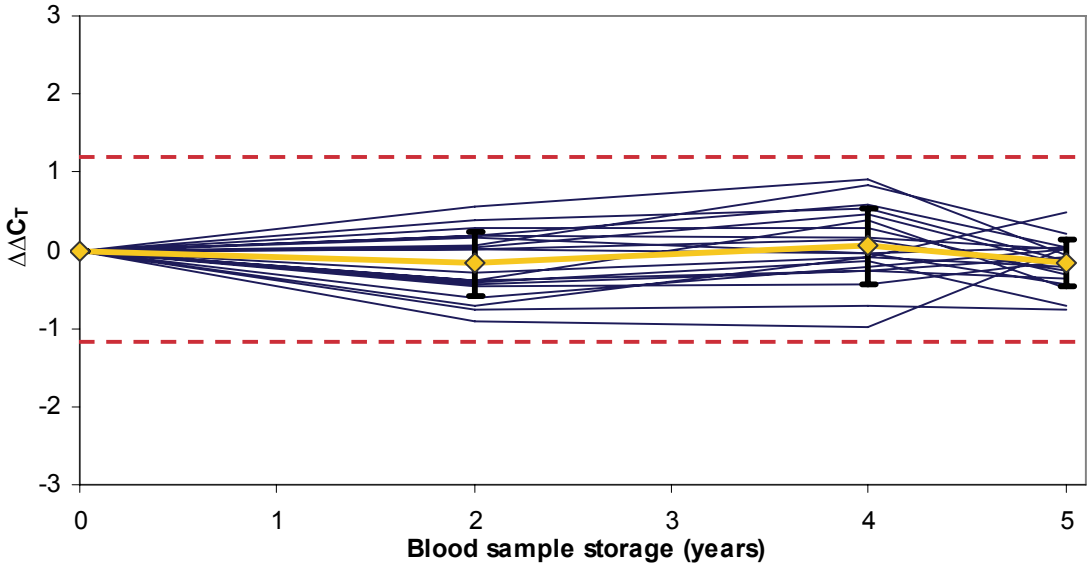

Figure 1. Relative transcript levels of FOS in RNA purified from blood stored in situ at –20°C in PAXgene Blood RNA Tubes. Blood was collected in duplicate from 10 donors. RNA was purified using the PAXgene Blood RNA Kit and analyzed using a quantitative RT-PCR assay specific for the FOS transcript. Mean values for all time points are plotted as a gold line with black vertical bars representing standard deviations. Assay precision (±3x total precision of the assay with consideration of single data; | 3 x σT | = 1.16 C_T) is shown as dashed red lines.

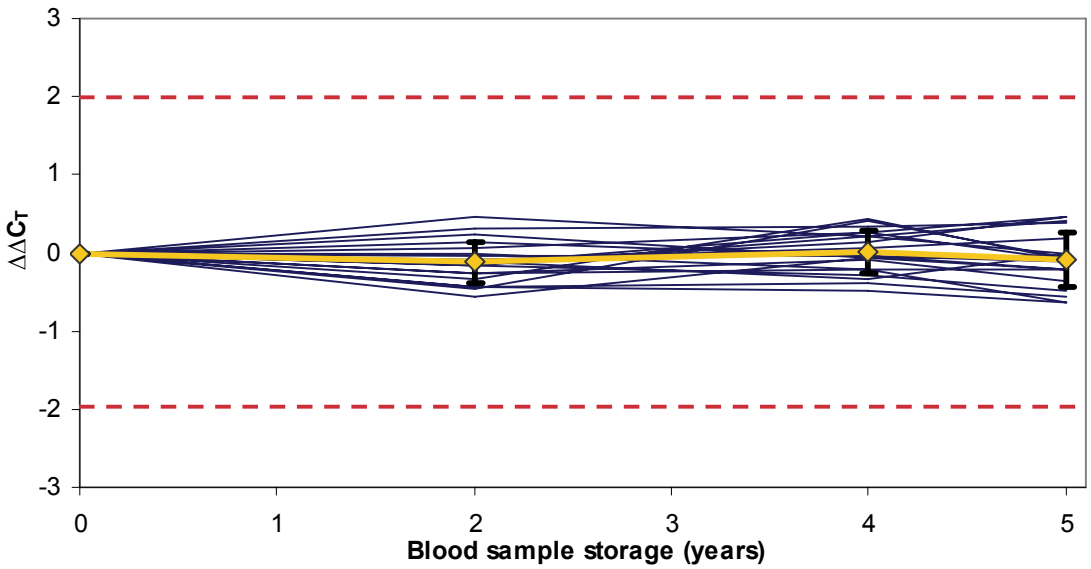

Figure 2. Relative transcript levels of IL1B in RNA purified from blood stored in situ at –20°C in PAXgene Blood RNA Tubes. Blood was collected in duplicate from 10 donors. RNA was purified using the PAXgene Blood RNA Kit and analyzed using a quantitative RT-PCR assay specific for the IL1B transcript. Mean values for all time points are plotted as a gold line with black vertical bars representing standard deviations. Assay precision (±3x total precision of the assay with consideration of single data; | 3 x σT | = 1.98 C_T) is shown as dashed red lines.

There were no significant changes in the relative transcript levels of FOS or IL1B due to in situ storage of whole blood in PAXgene Blood RNA Tubes at –20°C for up to 5 years (60 months). All variations in the $\Delta\Delta C_T$ values remained within the range of ±3x the total precision of the assay with consideration of single data (FOS: | 3 x σT | = 1.16 C_T; IL1B: | 3 x σT | = 1.98 C_T).

Figure 3 shows the RNA integrity numbers (RINs) for RNA purified from blood stored in PAXgene Blood RNA Tubes at –20°C for 5 years (60 months). After the indicated blood storage times, duplicate blood samples from 10 donors were processed using the PAXgene Blood RNA Kit.

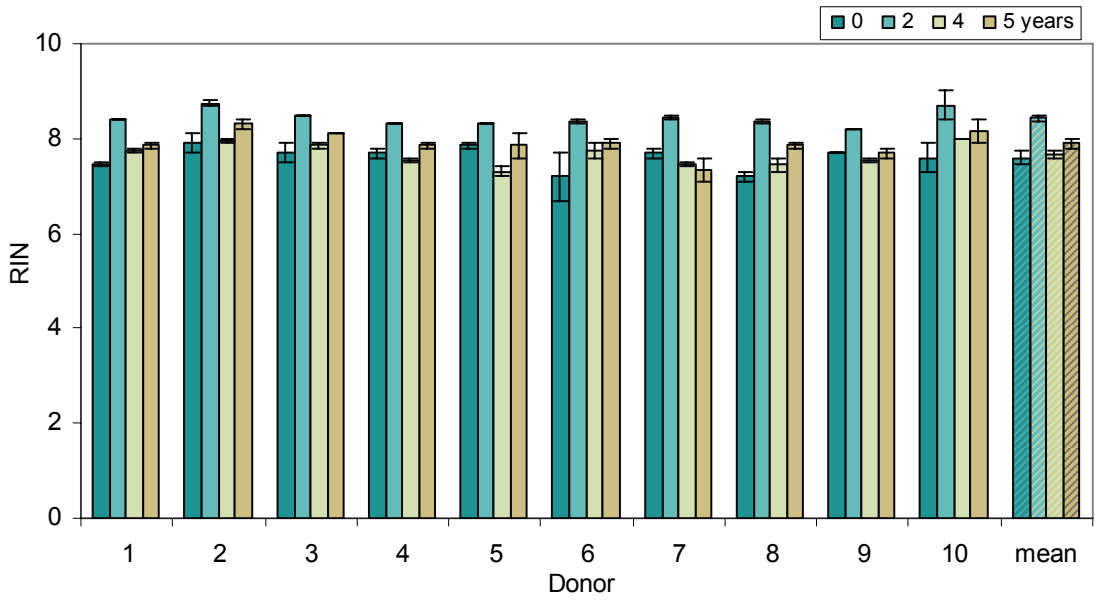

Figure 3. Integrity of RNA purified from whole blood stored in situ in PAXgene Blood RNA Tubes at –20°C. Mean RIN values for duplicate donor samples (**1–10**) and for all samples in total (**mean**) are shown for the indicated storage times. The error bars indicate the upper and lower RIN values of duplicate samples from an individual donor or, for the mean column, the standard deviations of RIN values of all samples from all donors.

No significant loss of RNA integrity was detected in whole blood samples stored for 5 years (60 months) at –20°C in PAXgene Blood RNA Tubes.

Stability of RNA in blood stored in situ at –70°C

Figures 4 and 5 show the change in relative FOS and IL1B transcript levels for RNA in blood stored in situ in PAXgene Blood RNA Tubes at –70°C.

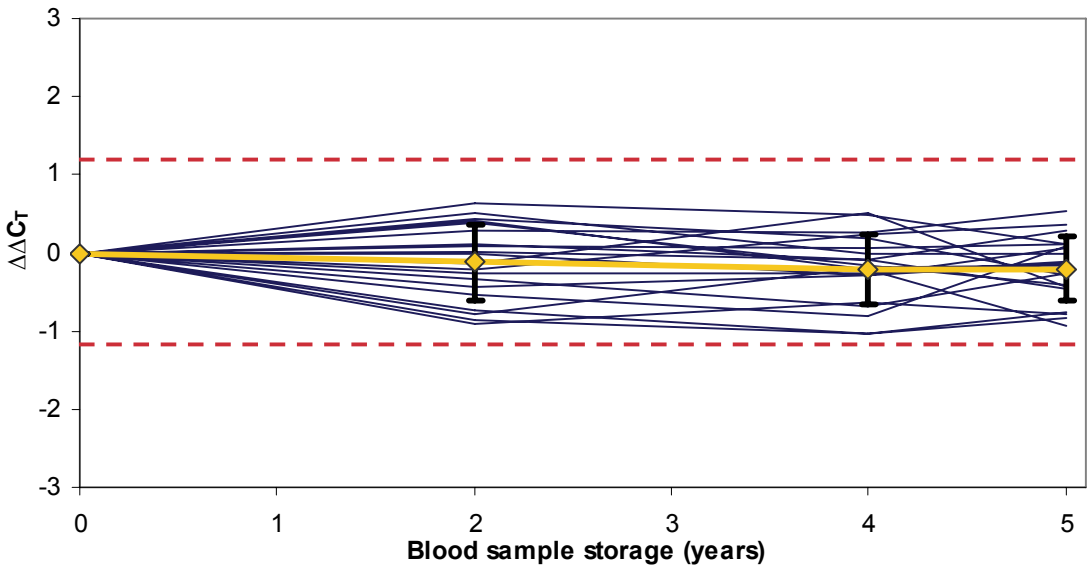

Figure 4. Relative transcript levels of FOS in RNA purified from blood stored in situ at –70°C in PAXgene Blood RNA Tubes. Blood was collected in duplicate from 10 donors. RNA was purified using the PAXgene Blood RNA Kit and analyzed using a quantitative RT-PCR assay specific for the FOS transcript. Mean values for all time points are plotted as a gold line with black vertical bars representing standard deviations. Assay precision (±3x total precision of the assay with consideration of single data; | 3 x σT | = 1.16 C_T) is shown as dashed red lines.

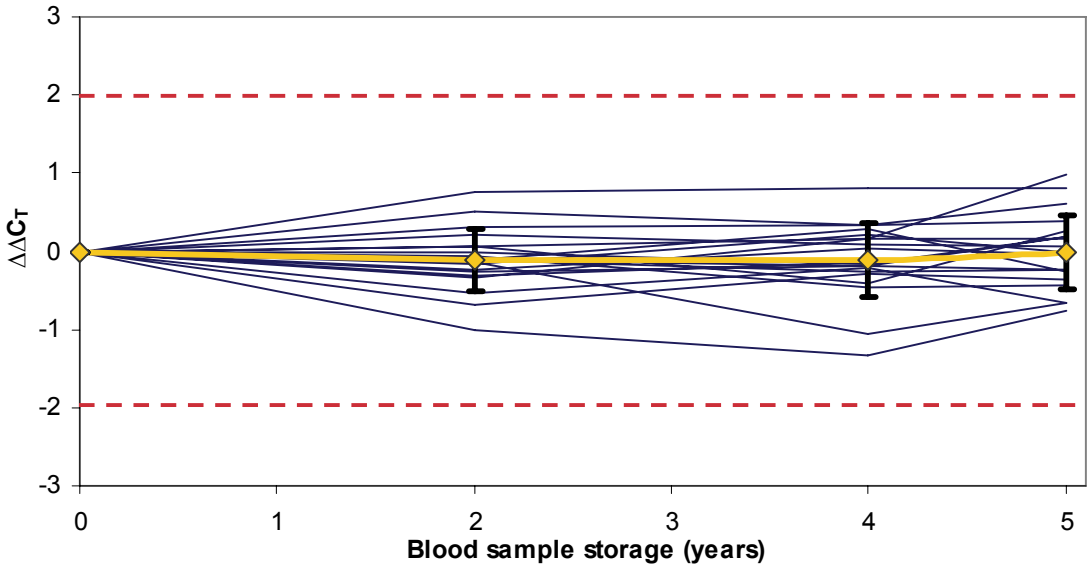

Figure 5. Relative transcript levels of IL1B in RNA purified from blood stored in situ at –70°C in PAXgene Blood RNA Tubes. Blood was collected in duplicate from 10 donors. RNA was purified using the PAXgene Blood RNA Kit and analyzed using a quantitative RT-PCR assay specific for the IL1B transcript. Mean values for all time points are plotted as a gold line with black vertical bars representing standard deviations. Assay precision (±3x total precision of the assay with consideration of single data; | 3 x σT | = 1.98 C_T) is shown as dashed red lines.

There were no significant changes in the relative transcript levels of FOS or IL1B due to in situ storage of whole blood in PAXgene Blood RNA Tubes at –70°C for up to 5 years (60 months). All variations in the $\Delta\Delta C_T$ values remained within the range of ±3x the total precision of the assay with consideration of single data (FOS: | 3 x σT | = 1.16 C_T; IL1B: | 3 x σT | = 1.98 C_T).

Figure 6 shows RNA integrity numbers (RINs) for RNA purified from blood stored in PAXgene Blood RNA Tubes at –70°C for 5 years (60 months). After the indicated blood storage times, duplicate blood samples from 10 donors were processed using the PAXgene Blood RNA Kit.

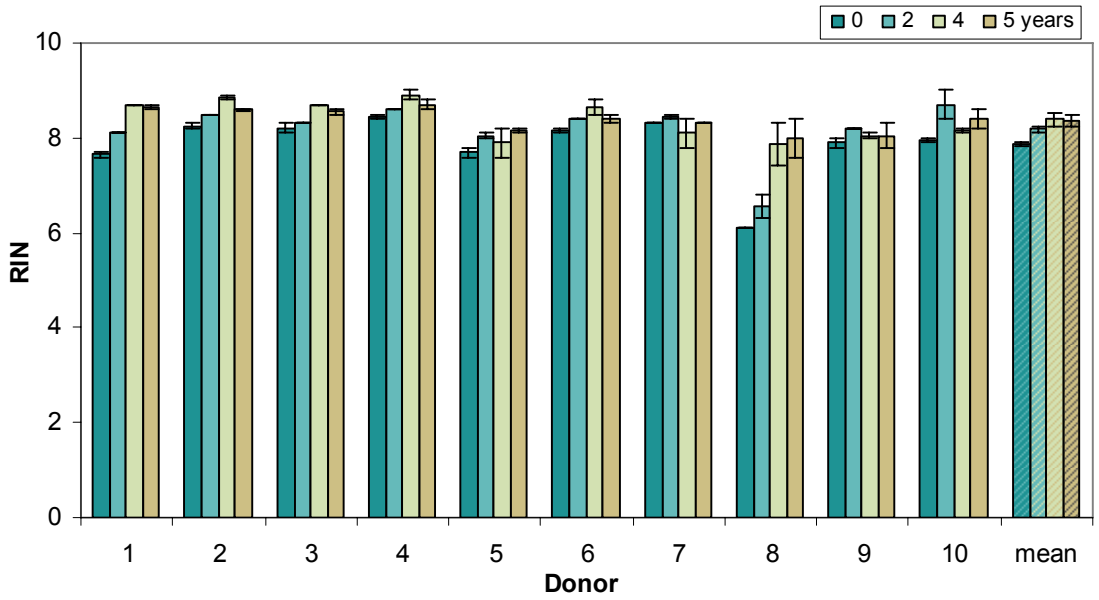

Figure 6. Integrity of RNA purified from whole blood stored in situ in PAXgene Blood RNA Tubes at –70°C. Mean RIN values for duplicate donor samples (**1–10**) and for all samples in total (**mean**) are shown for the indicated storage times. The error bars indicate the upper and lower RIN values of duplicate samples from an individual donor or, for the mean column, the standard deviations of RIN values of all samples from all donors.

No significant loss of RNA integrity was detected in whole blood samples stored for 5 years (60 months) at –70°C in PAXgene Blood RNA Tubes.

Conclusion

Blood can be stored in situ in PAXgene Blood RNA Tubes for at least 5 years (60 months) at either –20°C or –70°C without loss of function in quantitative RT-PCR analysis.

Furthermore, supplementary data indicated that, for measurements of blood from multiple donors stored in PAXgene Blood RNA Tubes before RNA preparation, mean RIN values were between 7 and 9 at all time points between zero and 5 years (60 months).

For up-to-date licensing information and product-specific disclaimers, see the respective PreAnalytiX® kit handbook or user manual. PreAnalytiX kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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