

Technical Note

PAXgene[®] Blood RNA System

In situ stability of RNA in blood specimens stored for 11 years (132 months) at -20°C^* and -70°C^* in PAXgene Blood RNA Tubes

Study Design

For each study, blood was collected into PAXgene Blood RNA Tubes from ten apparently healthy, consented adult subjects with white blood cell (WBC) counts in normal range ($4.8\text{--}11.0 \times 10^6$ WBC/ml blood). For each examined time point, blood was collected in quadruplicate. Two specimens were then stored in situ at -20°C and the other two at -70°C . Duplicate tubes from each subject were processed at the indicated time points[†] following the manual protocol in the PAXgene Blood RNA Kit Handbook.

The integrity of purified RNA was analyzed by capillary gel electrophoresis on an Agilent[®] Bioanalyzer 2100 instrument using RNA 6000 Nano reagents and chips[‡]. In addition, relative amounts of transcripts FOS and IL1B were determined by real-time quantitative RT-PCR on an ABI PRISM[®] 7900HT Sequence Detection System.

Results

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

Figures 1 and 2 show the change in relative transcript levels of FOS and IL1B, respectively, measured in blood stored in situ in PAXgene Blood RNA Tubes at -20°C . Over the course of 11 years (132 months) of in situ storage of whole blood in PAXgene Blood RNA Tubes at -20°C , relative transcript levels of FOS and IL1B remained constant, exhibiting no significant changes. All variations in the $\Delta\Delta\text{C}_T$ values remained within the range of $\pm 3\times$ the total precision of the assay (FOS: $|3 \times \sigma| = 1.16 \text{ C}_T$; IL1B: $|3 \times \sigma| = 1.98 \text{ C}_T$).

* Samples were stored at -20°C ($+5^{\circ}\text{C}/-10^{\circ}\text{C}$) and -70°C ($+5^{\circ}\text{C}/-20^{\circ}\text{C}$), which is the higher end of the temperature range for typical laboratory freezers (-20°C to $-30^{\circ}\text{C}/-40^{\circ}\text{C}$ or -70°C to $-80^{\circ}\text{C}/-86^{\circ}\text{C}$). Storage at the lower end of the temperature ranges is not expected to be detrimental to samples.

[†] This study is continuing.

[‡] RNA integrity results provided for information only; no claims for RNA integrity are made for the PAXgene Blood RNA System.

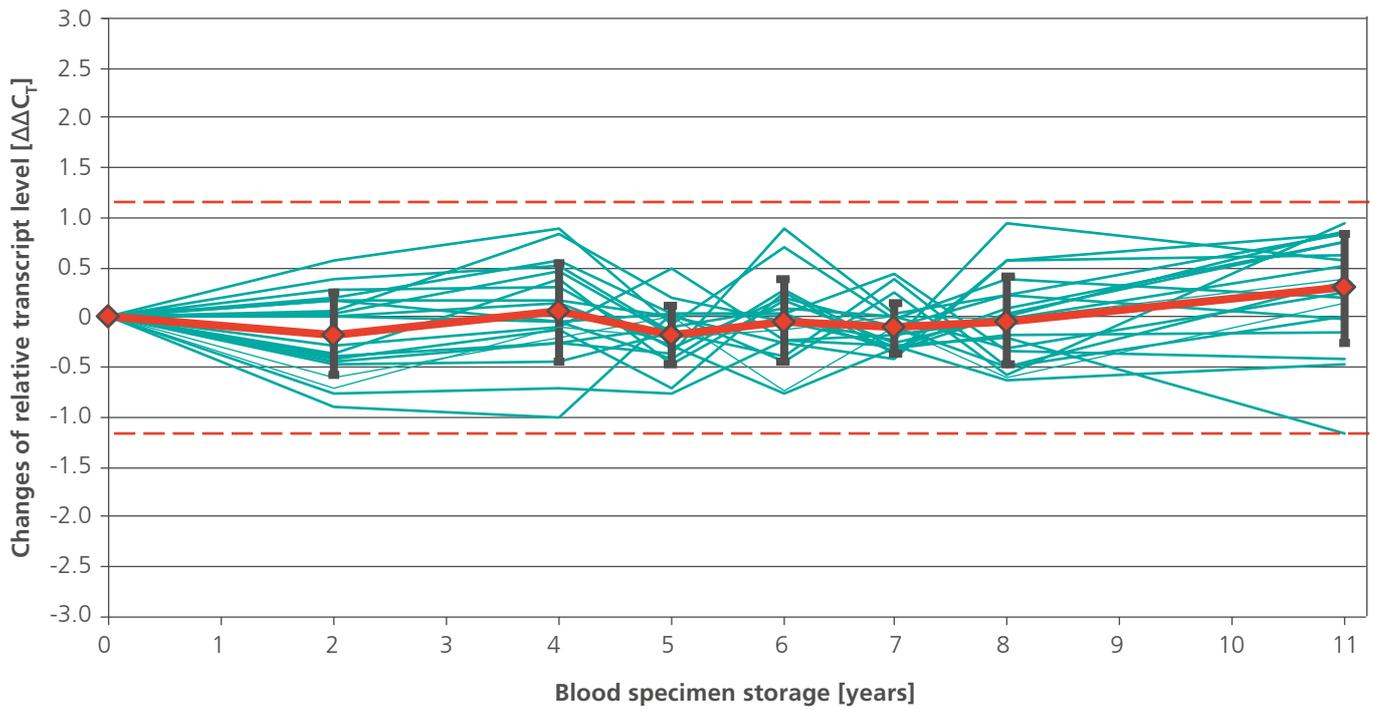


Figure 1. Relative transcript level changes of FOS in blood stored in situ at -20°C in PAXgene Blood RNA Tubes. Blood collected in duplicate from each of ten subjects was stored at -20°C . At each indicated time point, RNA was purified from duplicate tubes with the PAXgene Blood RNA Kit and analyzed using a quantitative RT-PCR assay specific for FOS. Mean $\Delta\Delta C_T$ values for all time points are plotted as a continuous red line with standard deviations. Red dashed lines represent assay precision ($\pm 3 \times$ total precision of the assay with consideration of single data; $|3 \times \sigma_T| = 1.16 C_T$).

Note: $\Delta\Delta C_T$ of test time point 11 years was calculated to data of test time point 8 years (set as reference) and not to data of test time point 0 (set as reference for all other test time points).

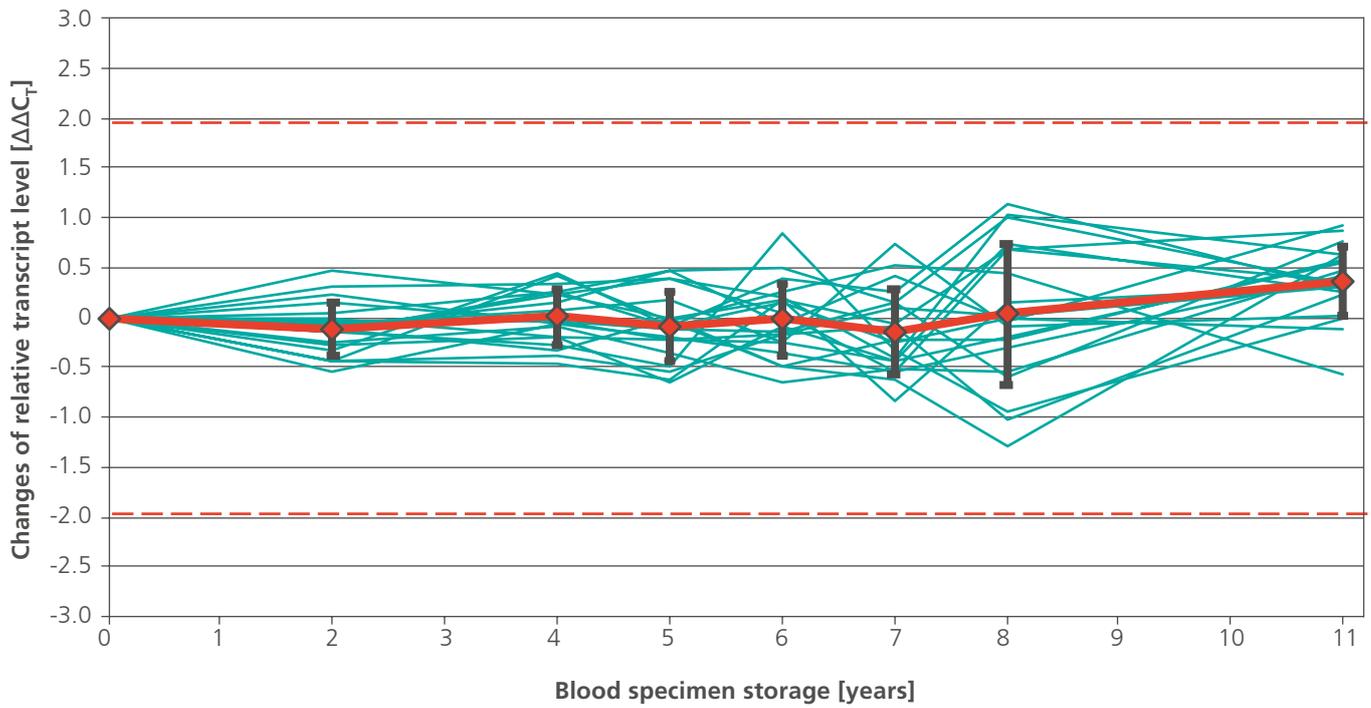


Figure 2. Relative transcript level changes of IL1B in blood stored in situ at -20°C in PAXgene Blood RNA Tubes. Blood collected in duplicate from each of ten subjects was stored at -20°C . At each indicated time point, RNA was purified from duplicate tubes with the PAXgene Blood RNA Kit and analyzed using a quantitative RT-PCR assay specific for IL1B. Mean $\Delta\Delta C_T$ values for all time points are plotted as a continuous red line with standard deviations. Red dashed lines represent assay precision ($\pm 3 \times$ total precision of the assay with consideration of single data; $|3 \times \sigma_T| = 1.98 C_T$).

Note: $\Delta\Delta C_T$ of test time point 11 years was calculated to data of test time point 8 years (set as reference) and not to data of test time point 0 (set as reference for all other test time points).

Figure 3 shows RNA integrity numbers (RINs) for RNA purified from blood stored in PAXgene Blood RNA Tubes at -20°C for 11 years (132 months). The purified RNA exhibited no significant loss of integrity.

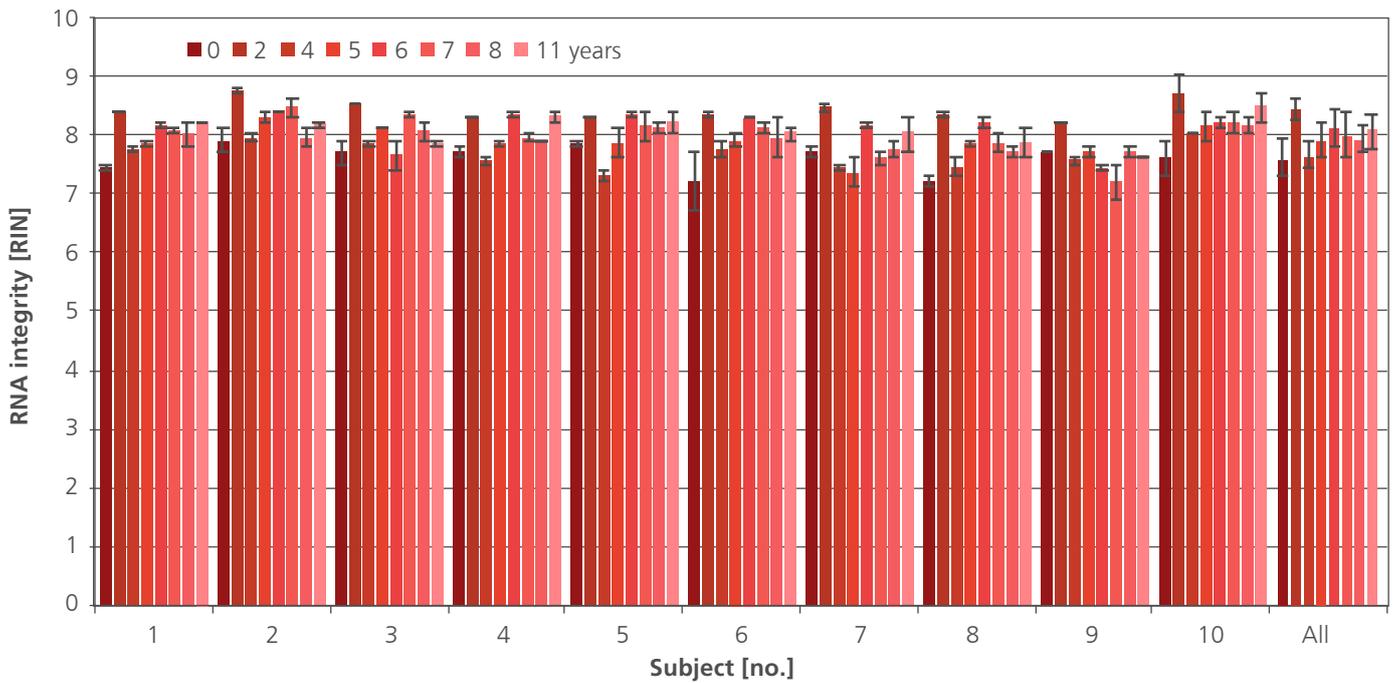


Figure 3. Integrity of RNA purified from blood stored in situ in PAXgene Blood RNA Tubes at -20°C . Mean RNA integrity number (RIN) scores for duplicate specimens (subjects 1–10) and for all specimens in total (mean) are given for the indicated storage times. The error bars indicate the upper and lower RIN scores of duplicate specimens from an individual subject or, for the mean column, the standard deviations of RIN scores of all specimens from all subjects.

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

Figures 4 and 5 illustrate the change in relative transcript levels of FOS and IL1B, respectively, measured in blood stored in situ in PAXgene Blood RNA Tubes at -70°C . 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 11 years (132 months). All variations in the $\Delta\Delta C_T$ values remained within the range of $\pm 3 \times$ the total precision of the assay with consideration of single data (FOS: $|3 \times \sigma| = 1.16 C_T$; IL1B: $|3 \times \sigma| = 1.98 C_T$).

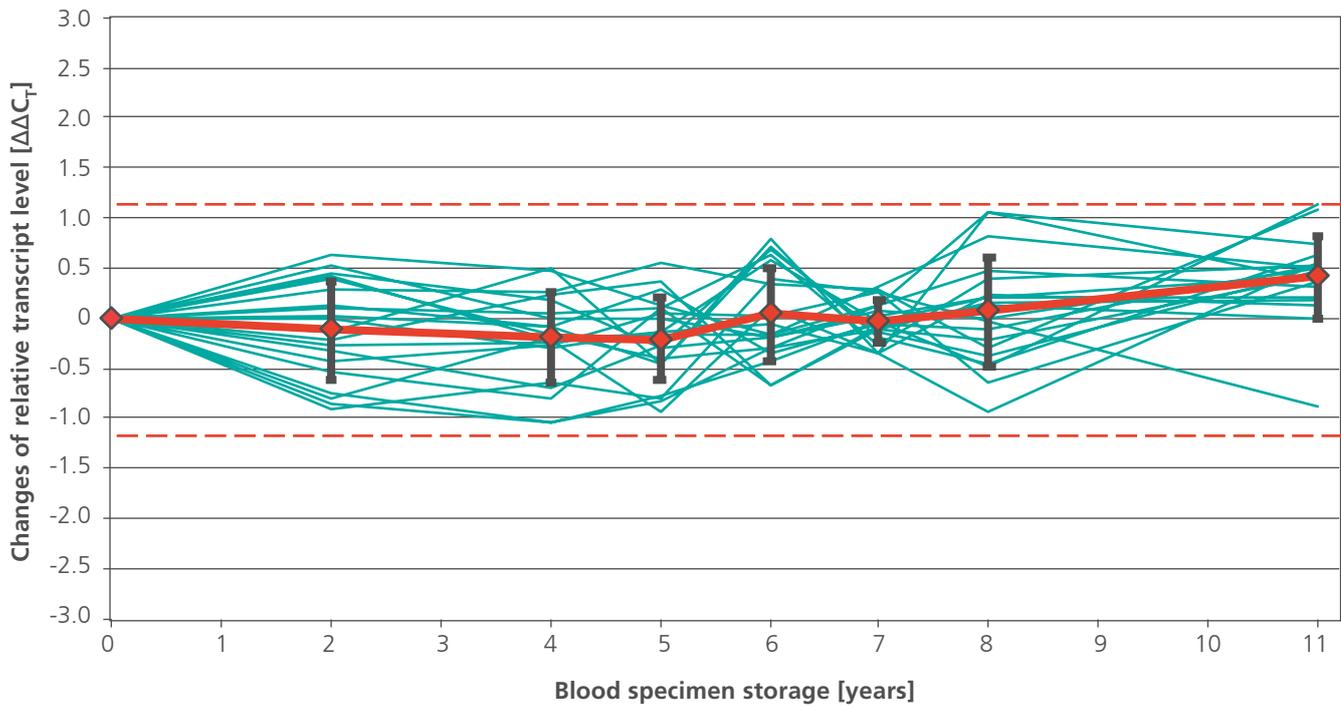


Figure 4. Relative transcript level changes of FOS in blood stored in situ at -70°C in PAXgene Blood RNA Tubes. Blood collected in duplicate from each of ten subjects was stored at -70°C . At each time point indicated, RNA was purified from duplicate tubes with the PAXgene Blood RNA Kit and analyzed using a quantitative RT-PCR assay specific for FOS. Mean $\Delta\Delta C_T$ values for all time points are plotted as a continuous red line with standard deviations. Red dashed lines represent assay precision ($\pm 3 \times$ total precision of the assay with consideration of single data; $|3 \times \sigma_T| = 1.16 C_T$).

Note: $\Delta\Delta C_T$ of test time point 11 years was calculated to data of test time point 8 years (set as reference) and not to data of test time point 0 (set as reference for all other test time points).

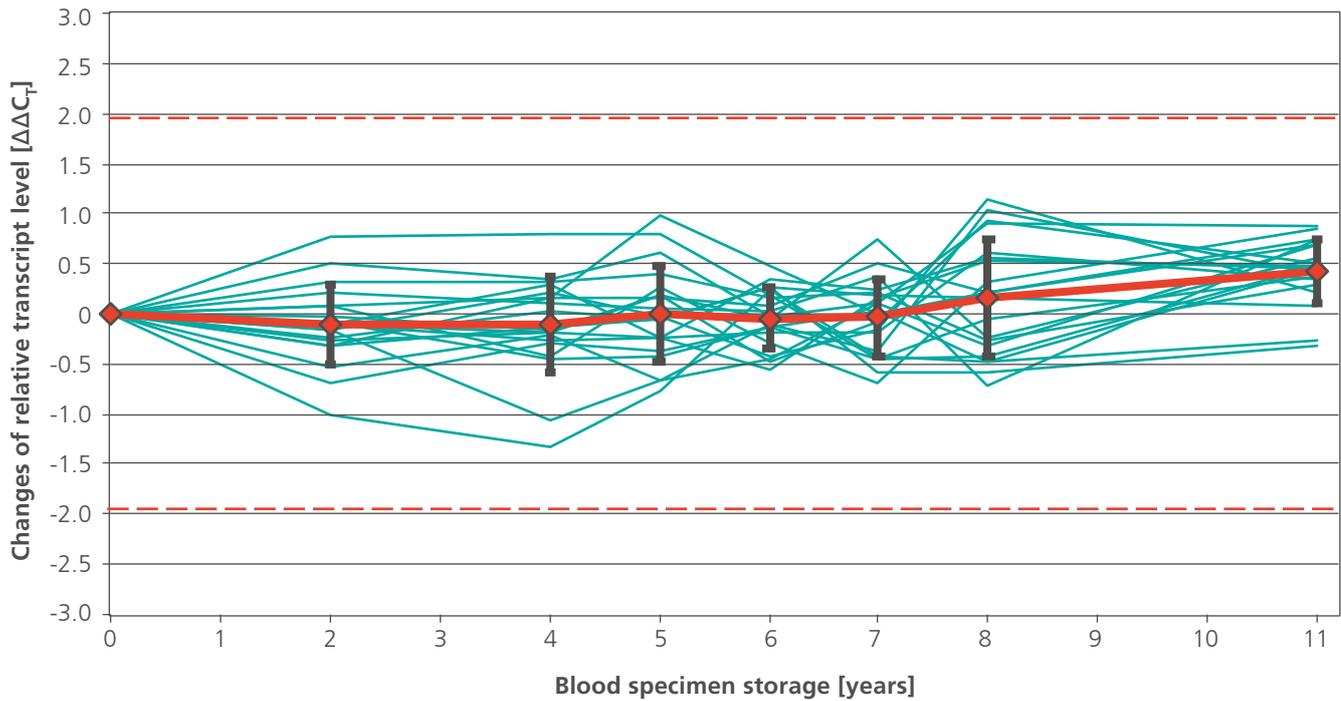


Figure 5. Relative transcript level changes of IL1B in blood stored in situ at -70°C in PAXgene Blood RNA Tubes. Blood collected in duplicate from each of ten subjects was stored at -70°C . At each time point indicated, RNA was purified from duplicate tubes with the PAXgene Blood RNA Kit and analyzed using a quantitative RT-PCR assay specific for IL1B. Mean $\Delta\Delta C_T$ values for all time points are plotted as a continuous red line with standard deviations. Red dashed lines represent assay precision ($\pm 3 \times$ total precision of the assay with consideration of single data; $|3 \times \sigma_T| = 1.98 C_T$).

Note: $\Delta\Delta C_T$ of test time point 11 years was calculated to data of test time point 8 years (set as reference) and not to data of test time point 0 (set as reference for all other test time points).

Figure 6 shows RNA integrity numbers (RINs) for RNA purified from blood stored in PAXgene Blood RNA Tubes at -70°C for 11 years (132 months). The purified RNA exhibited no significant loss of integrity.

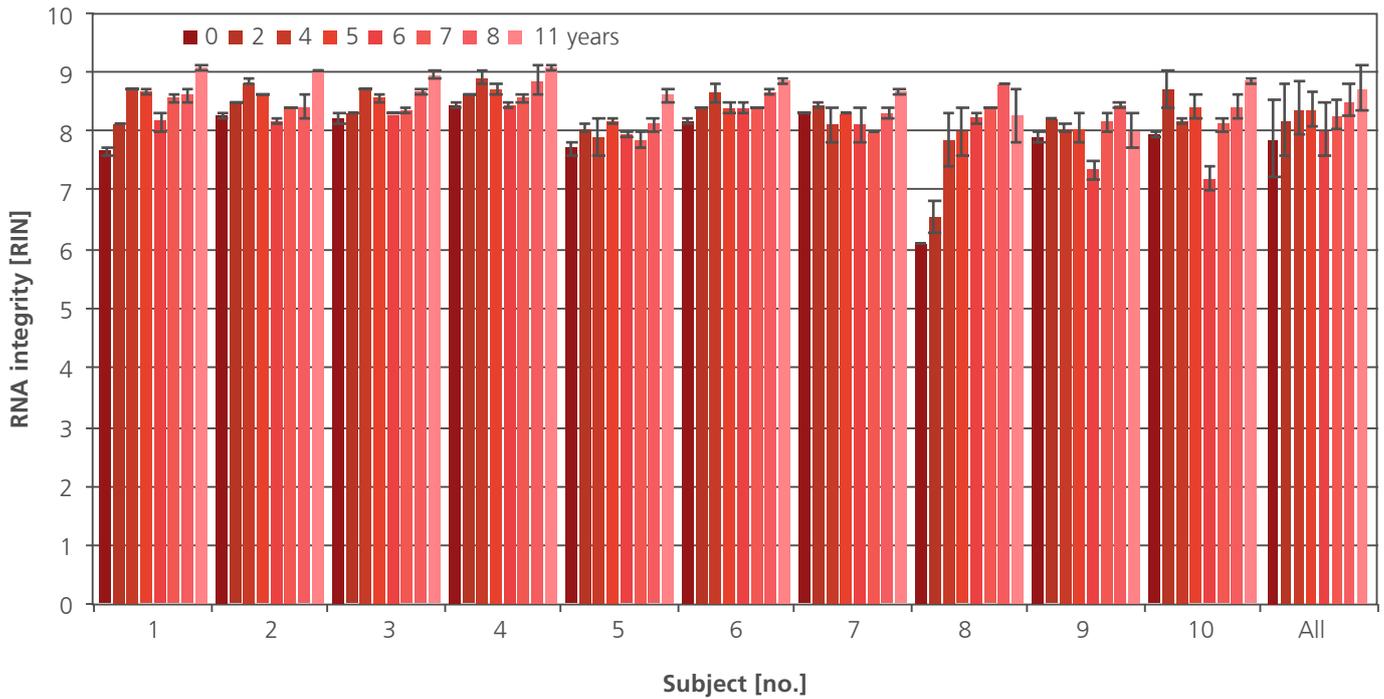


Figure 6. Integrity of RNA purified from blood stored in situ in PAXgene Blood RNA Tubes at -70°C . Mean RNA integrity number (RIN) scores for duplicate specimens (subjects 1–10) and for all specimens in total (mean) are shown for the indicated storage times. The error bars indicate the upper and lower RIN scores of duplicate specimens from an individual subject or, for the mean column, the standard deviations of RIN scores of all specimens from all subjects.

Conclusion

Blood can be stored in situ in PAXgene Blood RNA Tubes for at least 11 years (132 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 subjects stored in PAXgene Blood RNA Tubes before RNA preparation, mean RIN scores ranged between 7.6 and 8.7 at all time points between zero and 11 years (132 months).

Products used

Product	Catalog No.
PAXgene Blood RNA Tubes (100)	762165
PAXgene Blood RNA Kit (50)	762164 (North America) 762174 (other countries)



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

Trademarks: PAXgene®, PreAnalytiX® (PreAnalytiX GmbH); QIAGEN® (QIAGEN Group); ABI PRISM® (Applied Biosystems, LLC); Agilent® (Agilent Technologies, Inc.).

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