Technical Note
PAXgene® Blood RNA System

In situ stability of RNA in blood specimens stored for 8 years (96 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 (4.8–11.0 × 10⁶ 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 8 years (96 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 ΔΔC_T values remained within the range of ±3× the total precision of the assay (FOS: |3 × σ| = 1.16 C_T; IL1B: |3 × σ| = 1.98 C_T).

* This study is continuing for 10 years.
† 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 ΔΔCt values for all time points are plotted as a red line with standard deviations. Red dashed lines represent assay precision (±3× total precision of the assay with consideration of single data; |3 × σT| = 1.16 Ct).

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 ΔΔCt values for all time points are plotted as a red line with standard deviations. Red dashed lines represent assay precision (±3× total precision of the assay with consideration of single data; |3 × σT| = 1.98 Ct).
Figure 3 depicts the RNA integrity numbers (RINs) for RNA purified from blood stored in PAXgene Blood RNA Tubes at –20°C for 8 years (96 months). The purified RNA exhibited no significant loss of integrity.

![Graph showing RNA integrity numbers over time for blood stored at –20°C.]

**Figure 3.** Integrity of RNA purified from blood stored in situ in PAXgene Blood RNA Tubes at –20°C. Mean RNA integrity numbers (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 8 years (96 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 ΔΔC_{t} values for all time points are plotted as a red line with standard deviations. Red dashed lines represent assay precision (±3× total precision of the assay with consideration of single data; |3 × σT| = 1.16 C_{t}).

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 ΔΔC_{t} values for all time points are plotted as a red line with standard deviations. Red dashed lines represent assay precision (±3× total precision of the assay with consideration of single data; |3 × 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 8 years (96 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.](image)

Mean 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 8 years (96 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 7.0 and 9.0 at all time points between zero and 8 years (96 months).
Products used

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog No.</th>
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<tr>
<td>PAXgene Blood RNA Tubes (100)</td>
<td>762165</td>
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<td>PAXgene Blood RNA Kit (50)</td>
<td>762164 (North America) 762174</td>
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