In situ Stability of RNA in Blood Samples Stored at -20°C and -70°C in PAXgene Blood RNA Tubes

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Abstract

Gene expression analysis in peripheral blood is important in molecular research and diagnostics, and erroneous results can be caused by *ex vivo* changes of expression patterns. We therefore developed the PAXgene® Blood RNA System, launched in the U.S. and Europe as an IVD, for the collection of whole blood and the stabilization and purification of total RNA. The PAXgene Blood RNA Tube is widely used to archive specimens for later gene expression analysis. The aim of these ongoing studies is to determine the stability of blood RNA in PAXgene Tubes stored at -20°C and -70°C.

For each study, blood was drawn into PAXgene tubes from ten consented donors. Specimens were stored *in situ* at either -20°C or -70°C and processed according to manufacturer's instructions using the PAXgene Blood RNA Kit. Purified RNA was analyzed for integrity using the Agilent Bioanalyzer and tested in CFOS and IL1B qRT-PCR assays.

Results: There were no significant changes in the relative transcript levels of CFOS or IL1B during *in situ* storage of whole blood in PAXgene Blood RNA Tubes at either -20°C or -70°C for up to 50 months. Furthermore, no significant loss of RNA integrity was detected in whole blood specimens stored for 50 months at either temperature.

Conclusions: Blood can be stored *in situ* in PAXgene Blood RNA Tubes for at least 50 months at -20°C or -70°C without loss of function in qRT-PCR analysis. Furthermore, supplementary data indicated that mean values for RINs were between 7 and 8 at all time points between zero and 50 months.

Study Design

For each study, blood was drawn into PAXgene tubes from a minimum of ten 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.

Purified RNA was analyzed for integrity† using the Agilent Bioanalyzer and tested in qRT-PCR assays for CFOS and IL1B.

*An additional 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 1A and 1B depict the change in relative CFOS and IL1B transcript number respectively for RNA in blood stored in situ in PAXgene Blood RNA Tubes at -20°C.

Figure 1A: Changes of CFOS Relative Transcript Level

Figure 1A: Relative transcript levels of CFOS in RNA purified from blood stored *in situ* at -20°C in PAXgene Blood RNA Tubes. Blood, collected from 10 donors, each with two RNA sample preparation replicates, was analyzed. The means and standard deviations of all storage test time points are plotted as orange lines with black vertical bars. Assay precision (±3 x total precision of the assay with consideration of single data (VVS = | 3 x σT | = 2.34 C_T)) is shown as dashed red lines.

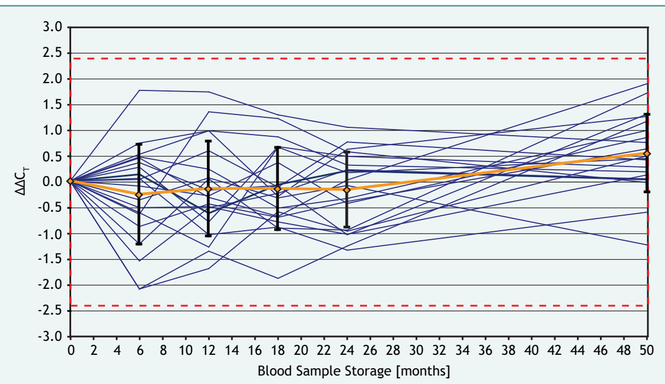
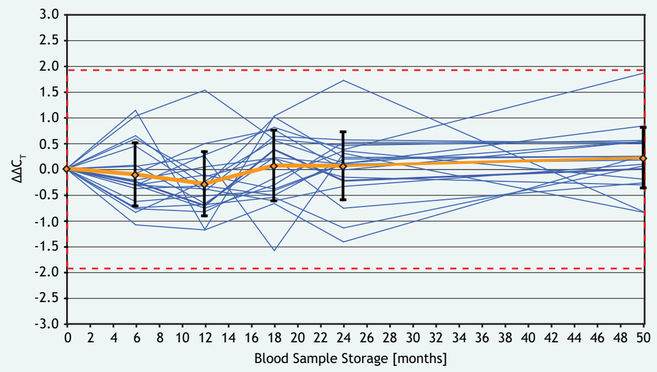


Figure 1B: Changes of IL1B Relative Transcript Level

Figure 1B: Relative transcript levels of IL1B in RNA purified from blood stored *in situ* at -20°C in PAXgene Blood RNA Tubes. Blood, collected from 10 donors, each with two RNA sample preparation replicates, was analyzed. The means and standard deviations of all storage test time points are plotted as orange lines with black vertical bars. Assay precision (±3 x total precision of the assay with consideration of single data (VVS = | 3 x σT | = 1.93 C_T)) is shown as dashed red lines.

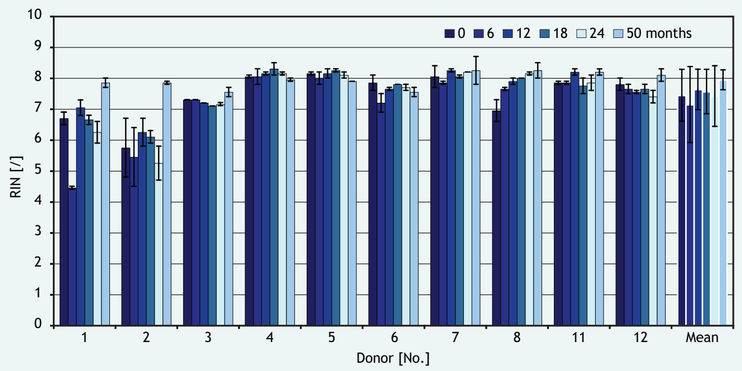


There were no significant changes in the relative transcript level of CFOS or IL1B due to *in situ* storage of whole blood in PAXgene Blood RNA Tubes at -20°C for up to 50 months. All variations in the $\Delta\Delta C_T$ values stayed within the range of ±3 x the total precision of the assay with consideration of single data (CFOS: VVS =| 3 x σ | = 2.34 C_T; IL1B: VVS = | 3 x σ | = 1.93 C_T).

Figure 2 depicts RNA integrity numbers (RINs) for RNA purified from blood stored in PAXgene Blood RNA Tubes at -20°C for 50 months. Using the PAXgene Blood RNA Kit, blood samples from 10 donors were processed in duplicate after the indicated blood storage times.

Figure 2: RNA Integrity

Figure 2: Integrity of RNA purified from whole blood stored in PAXgene Blood RNA Tubes at -20°C. Means of RINs of duplicates of all samples from all donors are presented as columns. The ends of upper and lower bars indicate the individual RINs of duplicate samples or, in the case of means, the standard deviations of RINs of all samples from all donors. Blood samples of donors 9 and 10 were excluded from study due to invalid WBC counts which were outside of the stated acceptance criteria.



No significant loss of RNA integrity was detected in whole blood samples stored for 50 months at -20°C in PAXgene Blood RNA Tubes.

Stability of RNA in Blood Stored *in situ* at -70°C

Figures 3A and 3B depict the change in relative CFOS and IL1B transcript number respectively for RNA in blood stored in situ in PAXgene Blood RNA Tubes at -70°C.

Figure 3A: Changes of CFOS Relative Transcript Level

Figure 3A: Relative transcript levels of CFOS in RNA purified from blood stored *in situ* at -70°C in PAXgene Blood RNA Tubes. Blood, collected from 10 donors, each with two RNA sample preparation replicates, was analyzed. The means and standard deviations of all storage test time points are plotted as orange lines with black vertical bars. Assay precision (±3 x total precision of the assay with consideration of single data (VVS = | 3 x σT | = 2.34 CT)) is shown as dashed red lines.

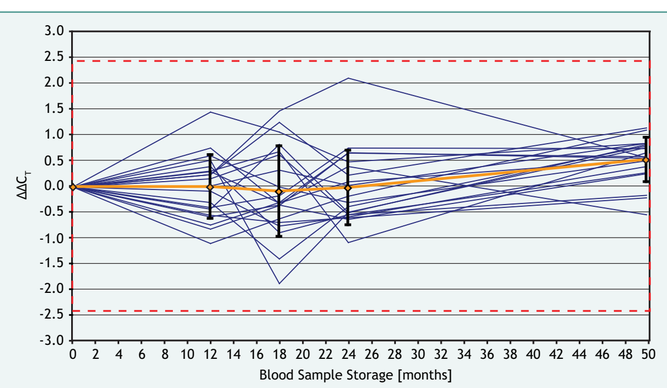
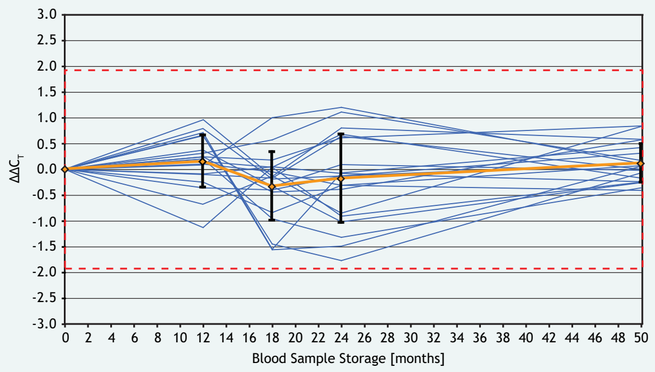


Figure 3B: Changes of IL1B Relative Transcript Level

Figure 3B: Relative transcript levels of IL1B in RNA purified from blood stored *in situ* at -70°C in PAXgene Blood RNA Tubes. Blood, collected from 10 donors, each with two RNA sample preparation replicates, was analyzed. The means and standard deviations of all storage test time points are plotted as orange lines with black vertical bars. Assay precision (±3 x total precision of the assay with consideration of single data (VVS = | 3 x σT | = 1.93 CT)) is shown as dashed red lines.

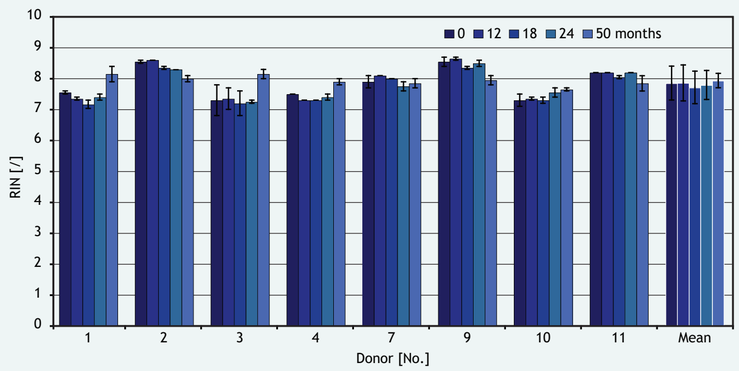


There were no significant changes in the relative transcript level of CFOS or IL1B due to *in situ* storage of whole blood in PAXgene Blood RNA Tubes at -70°C for up to 50 months. All variations in the $\Delta\Delta C_T$ values stayed within the range of ±3 x the total precision of the assay with consideration of single data (CFOS: VVS = | 3 x σ | = 2.34 CT; IL1B: VVS = | 3 x σ | = 1.93 C_T).

Figure 4 depicts RNA integrity numbers (RINs) for RNA purified from blood stored in PAXgene Blood RNA Tubes at -70°C for 50 months. Using the PAXgene Blood RNA Kit, blood samples from 10 donors were processed in duplicate after the indicated blood storage times.

Figure 4: RNA Integrity

Figure 4: Integrity of RNA purified from whole blood stored in PAXgene Blood RNA Tubes at -70°C. Means of RINs of duplicates of all samples from all donors are presented as columns. The ends of upper and lower bars indicate the individual RINs of duplicate samples or, in the case of means, the standard deviations of RINs of all samples from all donors. Blood samples of donor 5 were excluded from study due to invalid WBC counts which were outside of the stated acceptance criteria. Data sets of donors 6 and 8 (not shown) were also excluded due to an increase in RIN values which were outside of the normal variability of this measurement. Based on a Dixon test (level of significance alpha = 1%), these higher than expected RIN values were identified as outliers and were most likely the results of user error. The sets of data containing outliers were therefore excluded from calculation and presentation.



No significant loss of RNA integrity was detected in whole blood samples stored for 50 months at -70°C in PAXgene Blood RNA Tubes.

Conclusion

IL1B and CFOS gene transcript levels remain stable in PAXgene Blood RNA Tubes for at least 50 months at -20°C or -70°C.

Furthermore, supplementary data indicated that, for duplicate measurements of multiple donors, mean values for RINs were between 7 and 8 at all time points between zero and 50 months.