IN SITU STABILITY OF RNA IN BLOOD SAMPLES STORED AT -20°C AND -70°C IN PAXgene BLOOD RNA TUBES

Guenther K, Balven-Ross H, Wyrich R, Rainen L
PreAnalytiX GmbH, Hombrechtikon, CH, and QIAGEN GmbH, Hilden, GER

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 consenting 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 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 10⁶ WBC/ml blood. Replicate specimens were stored in situ at either -20°C or -70°C and processed in duplicate at the indicated blood storage times 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. 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.

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 1A: Changes of CFOS Relative Transcript Level

Figure 1B: Changes of IL1B Relative Transcript Level

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.

Figure 3A: Changes of CFOS Relative Transcript Level

Figure 3B: Changes of IL1B Relative Transcript Level

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

Figure 4: RNA Integrity

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.