



Technical Note PAXgene® Blood DNA Tube (IVD)

In situ stability of DNA in blood specimens stored for 1 year (12 months)
at –20°C in PAXgene Blood DNA Tubes (IVD)

Study Design

Blood was collected into PAXgene Blood DNA Tubes (IVD)* from 12 apparently healthy, consented adult subjects (white blood cell (WBC) counts of $4.7\text{--}11.3 \times 10^6$ WBC/ml blood). For every subject, venous whole blood was collected using standard phlebotomy procedure into replicate PAXgene Blood DNA Tubes at T_0 . One tube from each subject was processed and analyzed at T_0 , and the remaining tubes were stored at –20°C. At the indicated time points, one stored tube from each subject was thawed, processed, and analyzed.†

For all DNA purifications, a 200 μl blood aliquot was processed with the QIAGEN QIAAsymphony DSP DNA Mini Kit (elution volume: 200 μl) on the QIAGEN QIAAsymphony SP instrument. Eluted DNA quantity and quality‡ were analyzed by UV spectrophotometry on a SpectraMax® Plus 384. Suitability of the purified DNA for use with molecular diagnostic testing methods was verified for SSP HLA typing with the Immucor LIFECODES® HLA EZ Type® (DRDQ Low Resolution) assay according to manufacturer's instructions, using standard gel electrophoresis with the pre-cast 2% w/v agarose gels included in the assay kit.

* Product is currently not available in the USA.

† This study is continuing for 10 years.

‡ DNA quantity (concentration) and quality (purity) were calculated based on A_{260} and A_{280} values, both corrected for background absorbance value (A_{320}) as DNA concentration = $A_{260} \times 50 \text{ ng}/\mu\text{l} \times \text{dilution factor}$ and DNA purity = $A_{260}/A_{280} = (A_{260} - A_{320})/(A_{280} - A_{320})$.

Results

Figures 1 and 2 show the concentration and purity of the DNA isolated from blood stored in situ in PAXgene Blood DNA Tubes (IVD) at -20°C . Over the course of 1 year (12 months), the concentration of resulting DNA eluates remained above the acceptable lower limit to qualify for use in the Immucor LIFECODES HLA EZ Type (DRDQ Low Resolution) assay ($\geq 12.5 \text{ ng}/\mu\text{l}$). DNA purity stayed within the range of highly pure DNA, defined as corrected purity values of 1.7 to 1.9 (values rounded).

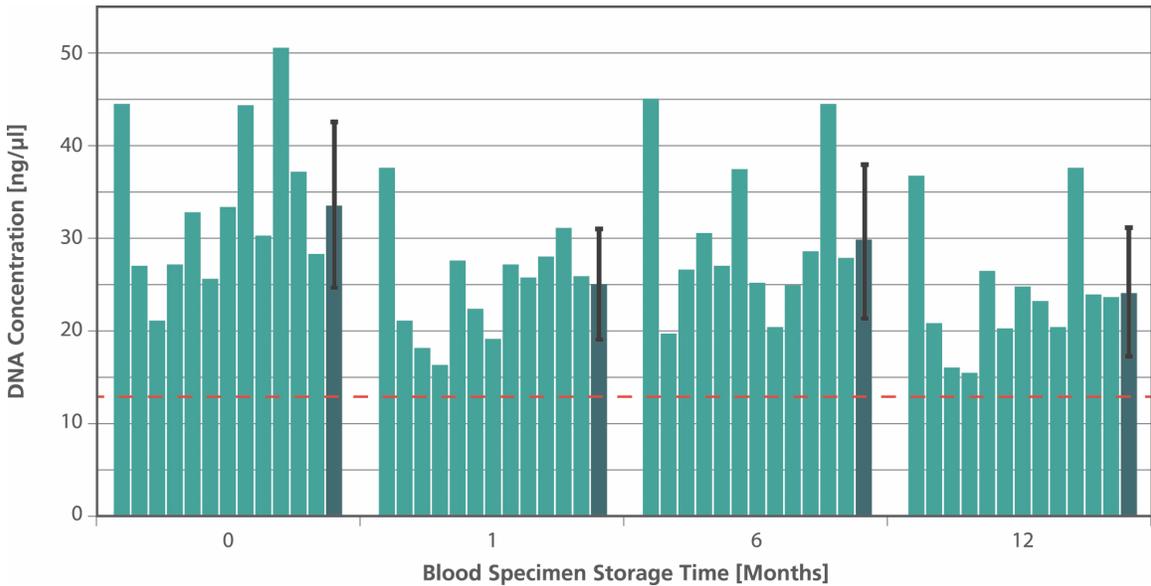


Figure 1. Eluate DNA concentrations from blood stored in situ at -20°C in PAXgene Blood DNA Tubes (IVD). Blood collected from each of 12 subjects was stored at -20°C . At each indicated time point, DNA was purified using the QIAasympyphony DSP DNA Mini Kit (blood input and DNA elution output volume: $200 \mu\text{l}$, each) on the QIAasympyphony SP instrument. DNA concentration was calculated based on A_{260} values corrected for background absorbance values (A_{320}), both obtained by UV spectrophotometry on a SpectraMax Plus 384 instrument. DNA concentrations of individual samples are plotted as solid bars in light turquoise. Mean DNA concentration for all specimens (subjects 1–12) at each time point appear in dark turquoise with standard deviation. The red dashed line indicates the minimum DNA concentration needed for further analysis of individual DNA samples with the Immucor LIFECODES HLA EZ Type (DRDQ Low Resolution) assay.

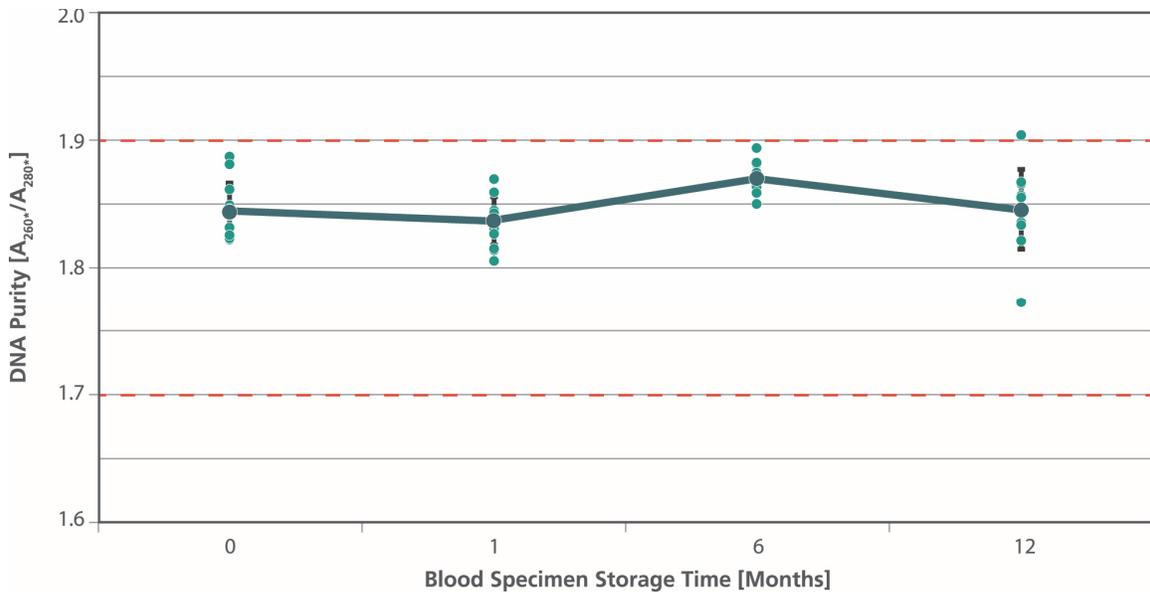


Figure 2. Eluate DNA purity from blood stored in situ at -20°C in PAXgene Blood DNA Tubes (IVD). DNA eluates from Figure 1 were additionally assayed for DNA purity. DNA purity was calculated based on absorbance values obtained by UV spectrophotometry on a SpectraMax Plus 384 instrument as A_{260^*}/A_{280^*} (where * indicates subtraction of background value A_{320}). DNA purity from individual samples are plotted as solid, light turquoise circles and mean DNA purity for all specimens (subjects 1–12) at each time point are depicted as connected dark turquoise circles with standard deviations. The red dashed lines represent the range of highly pure DNA, defined as corrected purity values of 1.7 to 1.9.

Figure 3 depicts representative PCR results obtained with the Immucor LIFECODES HLA EZ Type (DRDQ Low Resolution) assay for DNA of one subject purified from blood stored in PAXgene Blood DNA Tubes (IVD) at -20°C for up to 1 year (12 months). The expected pattern of allele positive and negative PCR results was obtained. The same held true for all tested samples. For each subject, the pattern of allele positive and negative PCR results obtained at each tested time point (1, 6 and 12 months) was identical to that obtained at T_0 .

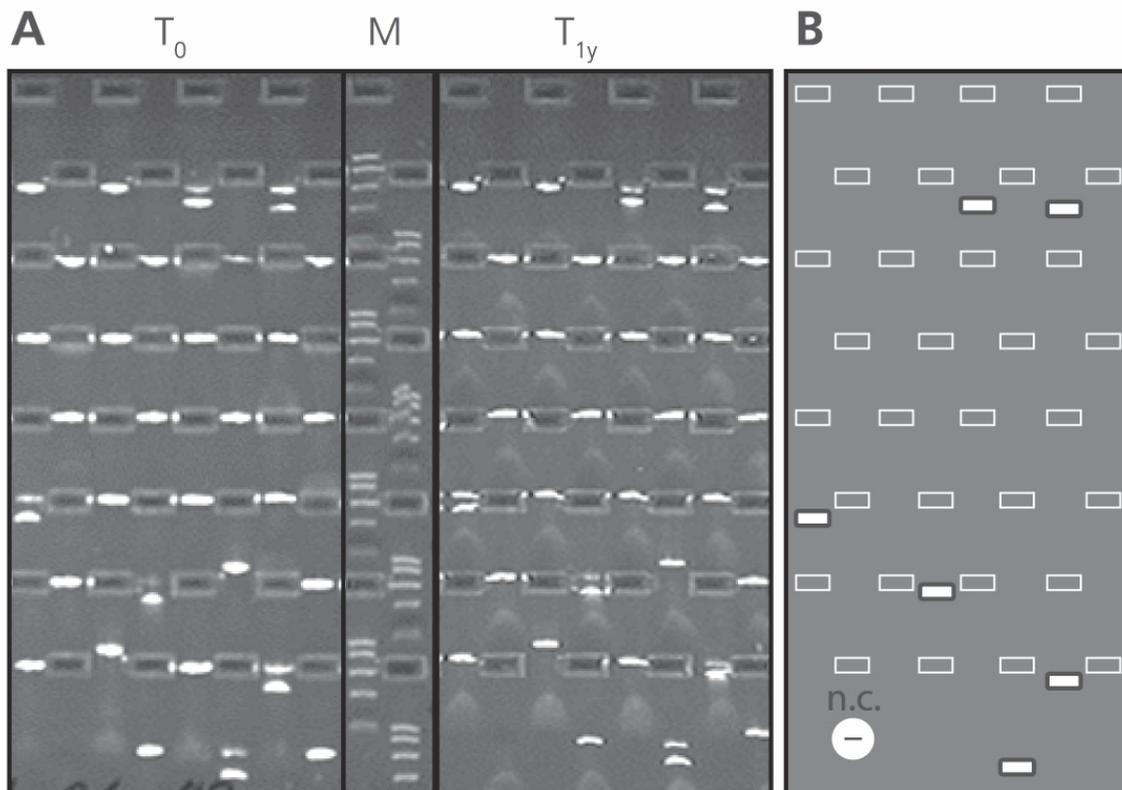


Figure 3. SSP HLA PCR results of DNA purified from blood stored in situ in PAXgene Blood DNA Tubes (IVD) at -20°C . (A) shows a representative electrophoretic gel result from DNA purified from blood collected into duplicate PAXgene Blood DNA Tubes (IVD) from one subject. One tube was processed immediately after phlebotomy (T_0) and one was stored at -20°C for 1 year (T_{1y}). (B) illustrates the concordant results of positive allele-specific PCRs in both DNA samples, indicated by white, black-outlined rectangles. The absence of PCR amplification products in the negative control reactions (n.c.) is indicated with a dash (-). M is the DNA molecular weight marker with fragment sizes of 1,500, 800, 400, 200, and 50 bp.

Conclusion

Blood can be stored in situ in PAXgene Blood DNA Tubes (IVD) for at least 1 year (12 months) at -20°C and the DNA purified from stored blood is still suitable for use in PCR genotyping assays, as exemplified by data obtained with the Immucor LIFECODES HLA EZ Type (DRDQ Low Resolution) assay.

Product used

Product	Catalog No.
PAXgene Blood DNA Tube (IVD) (1000)	761165

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.

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