

# **Performance of the PAXgene® Blood DNA Tube for the Collection, Transport, and Storage of Whole Blood and the Purification of DNA Using the QIASymphony® Instrument**

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## **ABSTRACT**

PreAnalytiX has developed a blood collection tube, the PAXgene Blood DNA Tube, for the collection of whole blood and the stabilization of genomic DNA. DNA is stable in whole blood stored in the PAXgene Blood DNA Tube for 28 days at 2-8°C, for 14 days at 18-25°C, for 3 days at 35°C, after 3 freeze/thaw cycles, or for a minimum of 6 months at -20°C. Blood can be frozen and thawed up to three times without appreciable loss of DNA concentration or quality. When tested in a molecular diagnostic assay, results from all DNA samples purified from whole blood collected and stored in the PAXgene Blood DNA Tube were concordant to results obtained from paired EDTA tube controls regardless of handling or storage condition. We have demonstrated that the PAXgene Blood DNA Tube can be used to collect and store whole blood specimens for the extraction of high quality DNA for use in molecular diagnostic test methods.

## **INTRODUCTION**

Blood collection tubes for molecular diagnostic test methods can vary with respect to anticoagulant, blood draw volume, and material composition (either glass or plastic). None of these tubes are cleared for general molecular diagnostic use for assays that require purified genomic DNA. Recently, we have developed the PAXgene Blood DNA Tube, a plastic, evacuated tube containing a proprietary ethylenediaminetetraacetic acid (EDTA) formulation which anticoagulates whole blood and preserves genomic DNA. The tube is designed to collect a nominal volume of 2.5 ml of blood upon venipuncture, and the blood can be stored in situ until it is needed for DNA extraction.

In this paper, we show the results of studies which were designed to demonstrate that the PAXgene Blood DNA Tube is compatible with an automated DNA extraction instrument and that it delivers DNA high quality DNA for use in molecular diagnostic test methods. Moreover, we show that the PAXgene Blood DNA Tube preserves genomic DNA for a minimum of 28 days at refrigerated temperature, 14 days at room temperature, 3 days at elevated temperature, after 3 freeze/thaw cycles, or at least 6 months frozen (study ongoing).

## **MATERIALS AND METHODS**

### **Subject Population**

All blood specimens were collected from apparently healthy, consented adult subjects.

### **Blood Collection and Specimen Processing**

Blood was collected into PAXgene Blood DNA Tubes (Cat. no. 761165, PreAnalytiX, Hombrechtikon, CH) and BD Vacutainer® Plus K<sub>2</sub>EDTA (EDTA) tubes (Cat. no. 367856, BD, Franklin Lakes, NJ, USA) using standard phlebotomy technique. Paired control (EDTA) and evaluation (PAXgene Blood DNA) tubes were collected from each subject and the evaluation tubes were stored at either room temperature (18-25°C), 2-8°C, 35°C, or frozen at -20°C. At designated time points, specimen aliquots were processed with the QIASymphony SP instrument (QIAGEN, Hombrechtikon, CH). Control tubes were processed on the day of collection without storage.

For Study 1 (See Figure 1), blood specimens were collected, and specimen aliquots (200 µl) were processed with the QIAGEN QIASymphony DSP DNA Mini Kit (QIAGEN, Hilden, DE; Cat. No. 937236). The resultant purified DNA was stored at -20°C prior to testing.

For Study 2 (See Table 1), blood specimens were collected and either processed on the day of blood collection (control and T<sub>0</sub> evaluation tubes) with the QIASymphony DSP DNA Mini Kit or stored at the indicated temperatures and processed using the same method at the designated time points (evaluation tube only).

### **DNA Concentration and Purity**

The concentration of DNA in QIASymphony eluates was determined by measuring the absorbances at 260 nm (A<sub>260</sub>) and 320 nm (A<sub>320</sub>) in a spectrophotometer and calculating the DNA concentration using the formula: µg of DNA/ml = [(A<sub>260</sub>) - (A<sub>320</sub>)] × 50 µg DNA/unit of absorbance.

DNA purity was determined by calculating the ratio of corrected absorbance at 260 nm to the corrected absorbance at 280 nm using the formula: [(A<sub>260</sub>) - (A<sub>320</sub>)] / [(A<sub>280</sub>) - (A<sub>320</sub>)]. This is the corrected A<sub>260</sub>/A<sub>280</sub> ratio. Pure DNA has a corrected A<sub>260</sub>/A<sub>280</sub> ratio of 1.7-1.9.

### **White Blood Cell Counts**

The number of white blood cells (WBCs) was counted from blood aliquots of EDTA tubes using a Beckman Coulter COULTER® Ac·T diff™ Analyzer.

### **Molecular Diagnostic Test**

Purified DNA aliquots from each specimen were tested in the Immucor (formerly Hologic Gen-Probe) LIFECODES® HLA EZ Type® (DRDQ Low Resolution) assay according to manufacturer's instructions.

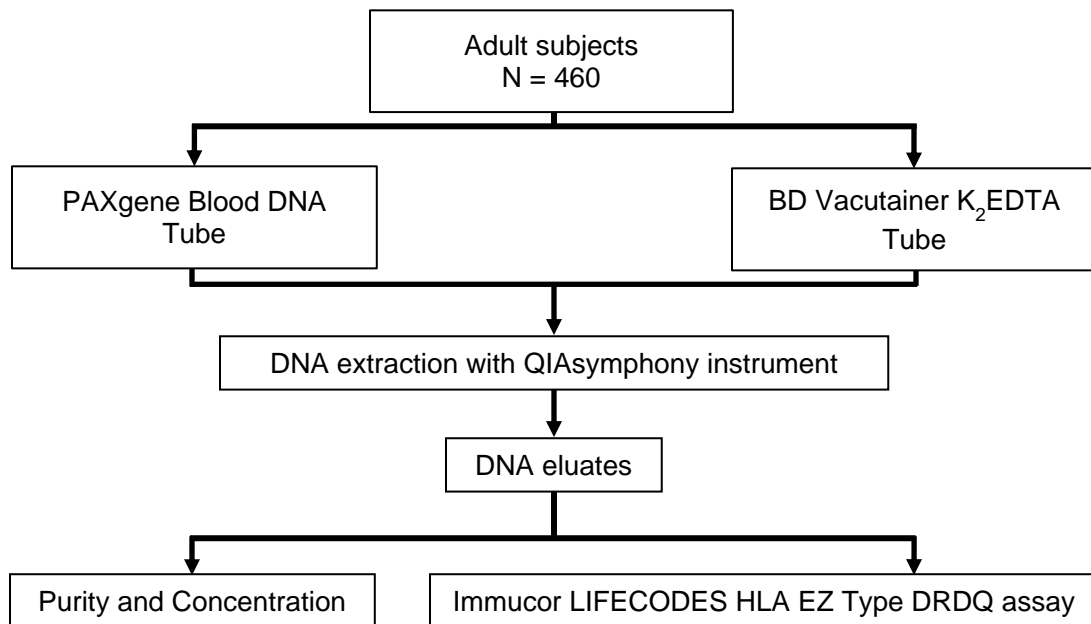
### **Data Analysis**

Median DNA eluate concentrations and the 25-75% interquartile range for DNA eluate concentrations were used as robust estimators of DNA quantity. All A<sub>260</sub>/A<sub>280</sub> ratios of purified DNA samples were rounded to one decimal point. An analysis of variance (ANOVA) was performed on the WBC vs. DNA eluate concentration to determine the relationship between these two values.

## Study Design

### Study 1: Quality, quantity, and suitability in molecular testing of DNA from whole blood collected in the PAXgene Blood DNA Tube and extracted with the QIASymphony instrument.

To demonstrate that DNA isolated from blood collected in the PAXgene Blood DNA Tube was of sufficient quality and quantity for use in molecular diagnostic test methods, venous whole blood was collected according to manufacturer's instructions from adult subjects into one PAXgene Blood DNA Tube (evaluation tube) and one BD Vacutainer Plus K<sub>2</sub>EDTA (control tube) tube. In total, 460 paired samples were collected. Within two days of phlebotomy, DNA was extracted from all specimens (200 µl) using the QIASymphony instrument and the QIASymphony DSP DNA Mini Kit. Purified DNA samples were assessed for purity and concentration as described in the Materials and Methods section and tested in the Immucor LIFECODES HLA EZ Type (DRDQ Low Resolution) assay according to the manufacturer's instructions. For study design, see Figure 1.



**Figure 1:** Study design of Study 1, “Quality, quantity, and suitability in molecular testing of DNA from whole blood collected in the PAXgene Blood DNA Tube and extracted with the QIASymphony instrument.”

## Study 2: Stability of DNA in whole blood stored in the PAXgene Blood DNA Tube.

For each storage or handling condition, a separate study was conducted to determine the stability of DNA in whole blood collected in the PAXgene Blood DNA Tube (See Table 1). For each study, venous whole blood from adult subjects was collected according to manufacturer's instructions into two EDTA tubes ( $T_0$  control) and one PAXgene Blood DNA Tube for each time point or handling condition (freeze/thaw study). The number of tubes collected per subject for each study was as follows: 6 tubes for room temperature (18-25°C) storage, 7 tubes for refrigerated temperature (2-8°C) storage, 7 tubes for elevated temperature (35°C) storage, 15 tubes for frozen (-20°C) storage. DNA from initial time point ( $T_0$ ) tubes was purified from whole blood using the QIA Symphony instrument on the day of collection. The quality and quantity of DNA extracted at each time point was assessed by spectrophotometry as described in the Materials and Methods section, and all purified DNA samples were tested in the Immucor LIFECODES HLA EZ Type (DRDQ Low Resolution) assay according to the manufacturer's instructions.

In addition to time/temperature handling studies, the performance of DNA from whole blood collected in PAXgene Blood DNA Tubes was determined when the specimen was subjected to multiple freeze/thaw cycles. For each subject, venous whole blood was collected according to manufacturer's instructions into two control tubes ( $T_0$  control) and four PAXgene Blood DNA tubes for a total of six tubes per subject. DNA was extracted with the QIA Symphony instrument from the two EDTA tubes and one PAXgene Blood DNA Tube on the day of collection, and the remaining three PAXgene Blood DNA Tubes were frozen at -20°C. After 24 hours, the three PAXgene tubes were thawed, DNA was extracted from one tube and the remaining two PAXgene tubes were frozen again at -20°C. After 24 hours, the two tubes were thawed, DNA was extracted from one tube, and the remaining tube was refrozen. The third tube was thawed after 24 hours and the DNA extracted.

Number of Subjects	Storage Temperature	Time Points/(Cycles)	Tubes per Subject
N = 12	Room Temperature (18-25°C)	$T_0$ , 3 d, 7 d, 14 d	6
N = 12	Refrigerated Temperature (2-8°C)	$T_0$ , 7 d, 14 d, 21 d, 28 d	7
N = 12	Elevated Temperature (35°C)	$T_0$ , 6 h, 1 d, 2 d, 3 d	7
N = 12	Frozen (-20°C)	$T_0$ , 1 m, 6 m, 12 m ...10 y	15
N = 12	Freeze/Thaw	$T_0$ , (1x, 2x, 3x)	6

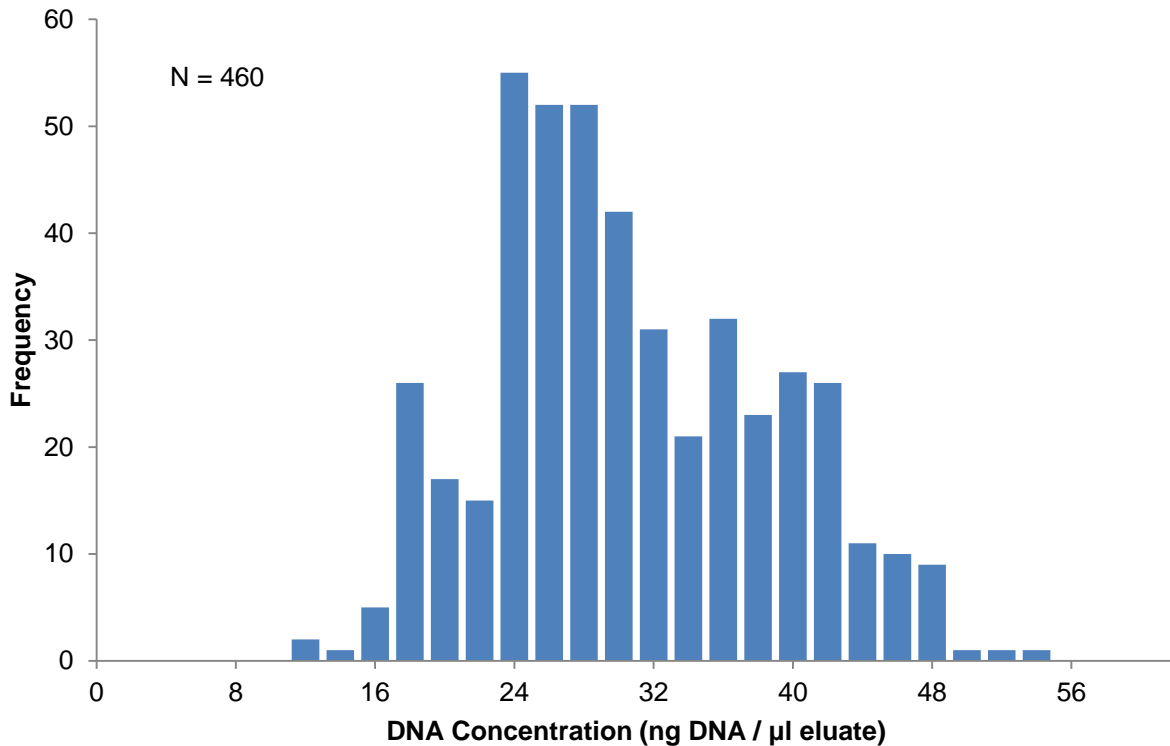
**Table 1:** Study design of Study 2, "Stability of DNA in whole blood collected in the PAXgene Blood DNA Tube."

All DNA isolated from samples used in this study were measured for DNA eluate concentration and purity as described in the Materials and Methods section and tested for performance in the Immucor LIFECODES HLA EZ Type (DRDQ Low Resolution) assay using an EDTA tube as control.

## RESULTS

### Study 1: Quality, quantity, and suitability in molecular testing of DNA from whole blood collected in the PAXgene Blood DNA Tube and extracted with the QIASymphony instrument.

A histogram of eluate concentrations obtained from 460 whole blood specimens collected and stored in PAXgene Blood DNA tubes is shown in Figure 2. DNA eluate concentrations ranged from 12.2 to 54.0 ng/μl with 75% of samples ≥ 24.8 ng/μl.



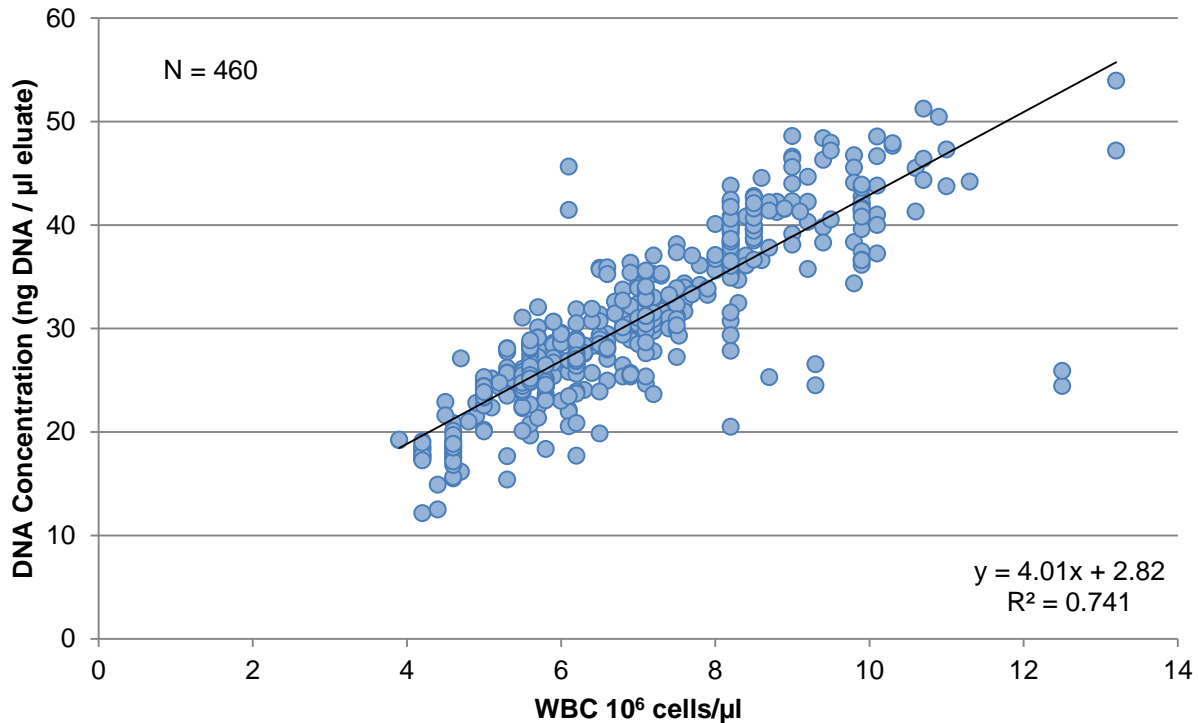
**Figure 2:** DNA concentration (ng DNA / μl eluate) in eluates of whole blood collected in PAXgene Blood DNA Tubes and purified using the QIASymphony instrument.

Median DNA concentrations, the 25-75% interquartile range, and the range of corrected  $A_{260}/A_{280}$  ratios of purified DNA samples are shown in Table 2.

Measurement	ng DNA / μl eluate ( $A_{260}/A_{280}$ ratio)
Median DNA eluate concentration	29.4
25-75% interquartile range	24.8-36.6
Range	12.2-54.0
DNA Purity	(1.7-1.9)

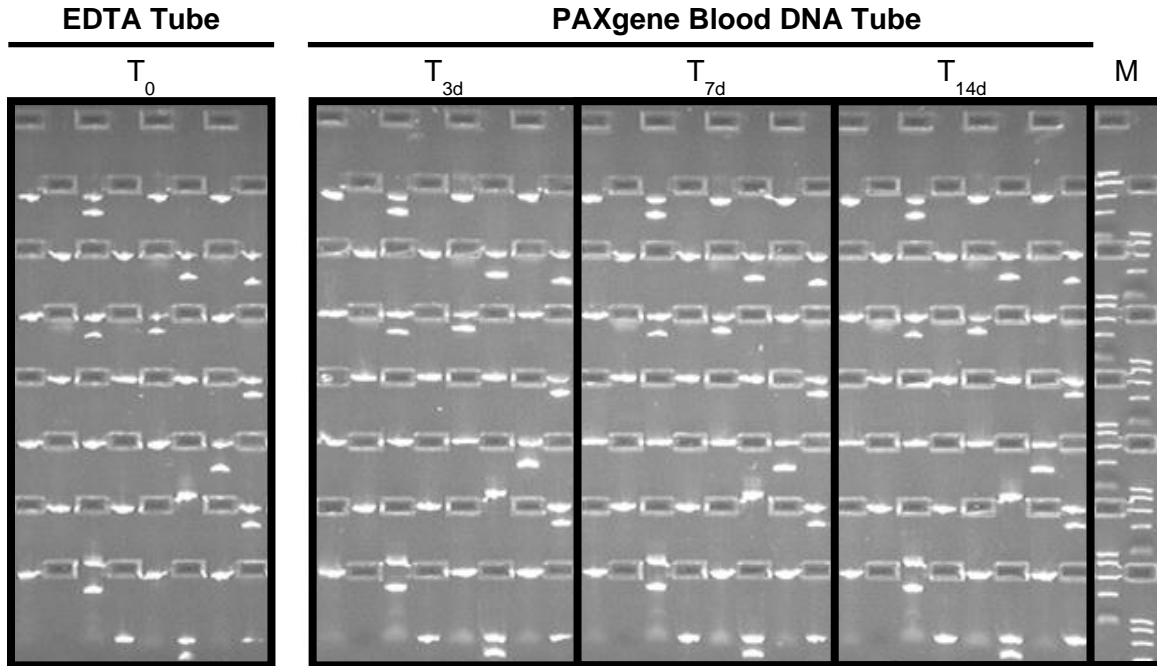
**Table 2:** DNA eluate concentrations, median, range, 25-75% interquartile range, and DNA purity from whole blood specimens collected in the PAXgene Blood DNA Tube and purified with the QIASymphony instrument.

DNA was successfully isolated from blood specimens with WBC ranging from  $3.9$  to  $13.2 \times 10^6$  cells/ $\mu\text{l}$  with no reported extraction failures. The correlation between WBC and DNA eluate concentration is depicted in Figure 3. An analysis of variance (ANOVA) of these results showed that the relationship between WBC and DNA concentration is statistically significant at an  $\alpha$ -level of  $0.05$  with a  $p$ -value of  $0.000$ .



**Figure 3:** WBC ( $10^6$  cells/ $\mu\text{l}$ ) vs. DNA concentration (ng DNA /  $\mu\text{l}$  eluate) in eluates of whole blood collected in PAXgene Blood DNA Tubes and purified using the QIAAsymphony instrument.

All purified DNA samples from blood collected in the PAXgene Blood DNA Tube were tested in the Immucor LIFECODES HLA EZ Type (DRDQ Low Resolution) assay and compared to results obtained from paired EDTA control tubes. Representative results of testing of extracted DNA in the EZ Type DRDQ HLA assay are shown in Figure 4. Individual patterns of HLA-SSP PCR amplicons which were separated by size on agarose gels were identical between DNA from specimens collected in the PAXgene Blood DNA Tubes and specimens collected in corresponding control EDTA tubes. HLA typing results ( $n = 110$ ) of blood collected in the PAXgene Blood DNA Tube were 100% concordant with results obtained with blood collected in the paired EDTA control tube.



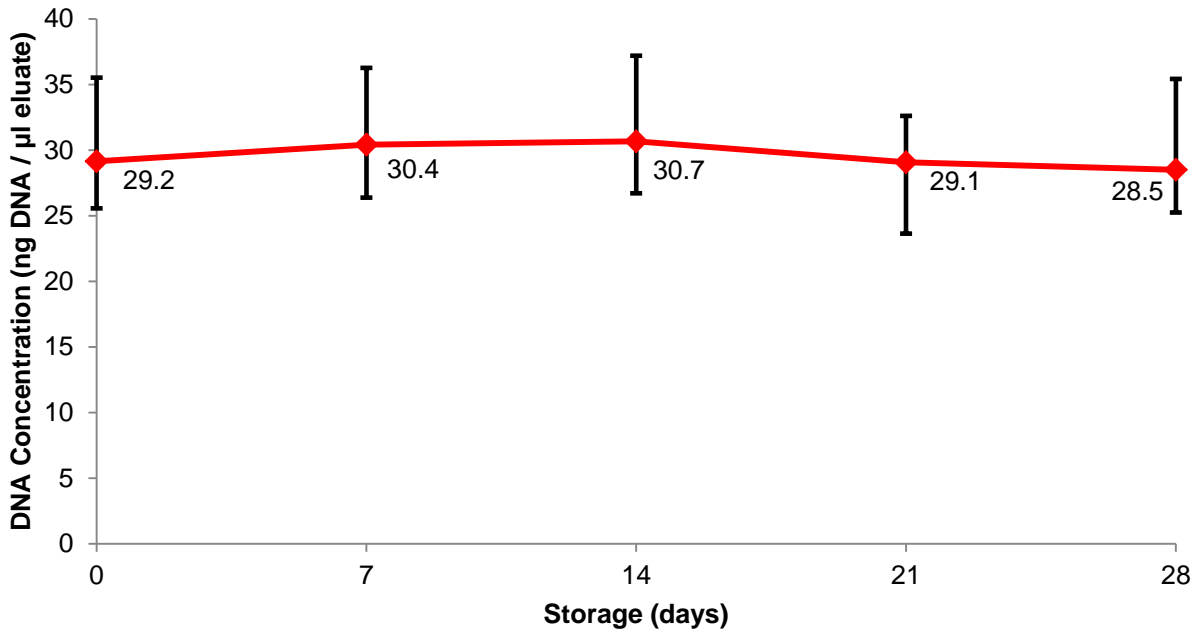
**Figure 4:** Electrophoretic gel results of the EZ Type DRDQ HLA assay showing HLA types from purified DNA from blood collected in paired EDTA and PAXgene Blood DNA Tubes. DNA was isolated from the control EDTA tube within two hours of phlebotomy ( $T_0$ ) and from PAXgene Blood DNA Tubes at 3 days ( $T_{3d}$ ), 7 days ( $T_{7d}$ ), and 14 days ( $T_{14d}$ ) after phlebotomy. (M = DNA molecular weight marker with fragment sizes of 1,500, 800, 400, 200, and 50 bp)

**Study 2: Quality, quantity and suitability for molecular diagnostic testing of blood stored in the PAXgene Blood DNA Tube.**

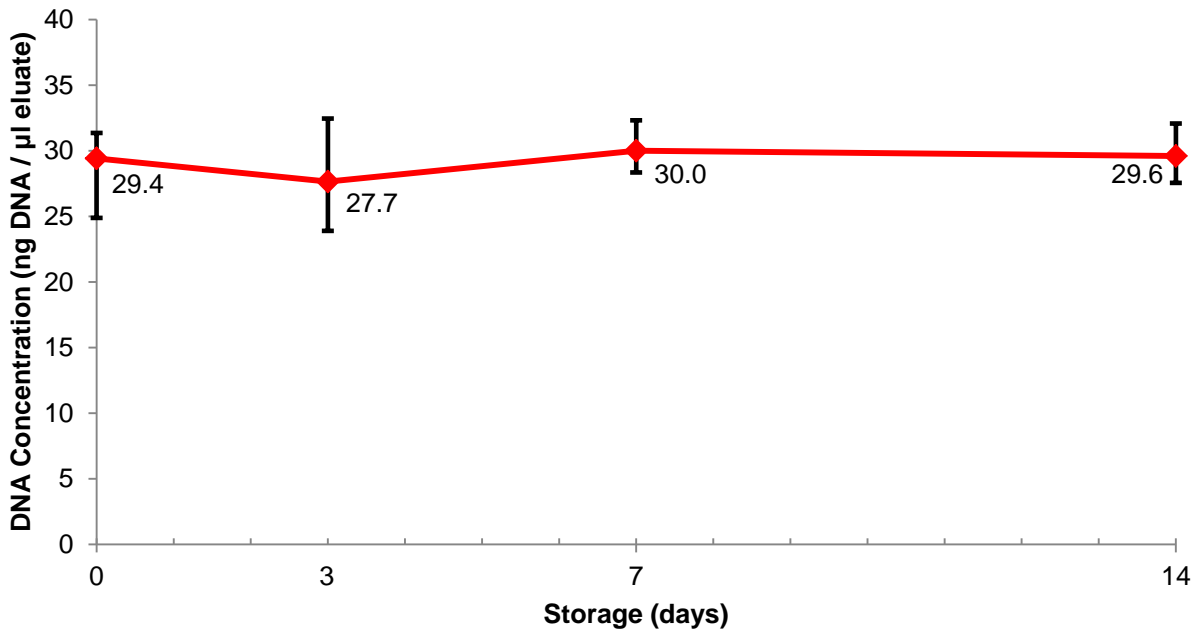
Results of the stability of DNA in whole blood stored at 2-8°C for 28 days, room temperature for 14 days, 35°C for 3 days, after 3 freeze/thaw cycles, or at -20°C for 6 months are shown in Figures 5, 6, 7, 8, and 9 respectively. DNA concentrations in the eluates are expressed in ng/μl.

In comparison to replicate tubes processed immediately after collection ( $T_0$ ), the median DNA concentration of the purified DNA eluates from blood stored at refrigerated (2-8°C) temperatures for 28 days was 28.5 ng/μl, or approximately 98% (Figure 5) while the median DNA eluate concentration in blood stored at room temperature for 14 days was 29.6 ng/μl, or 100% of the  $T_0$  eluate concentration (Figure 6). Median DNA eluate concentrations for blood stored for 3 days at 35°C was 26.2 ng/μl or 84% of the median  $T_0$  concentration (Figure 7). After three freeze/thaw cycles, the median DNA eluate concentration was 91% of that of DNA extracted from unfrozen whole blood (Figure 8). For all time/temperature or freeze/thaw storage conditions tested, DNA purified from all specimens remained of high quality with corrected  $A_{260}/A_{280}$  ratios between 1.7 and 1.9 (data not shown).

Blood stored in the PAXgene Blood DNA Tube at  $-20^{\circ}\text{C}$  was tested for DNA eluate concentration and purity. Median DNA eluate concentrations for blood stored for 6 months at  $-20^{\circ}\text{C}$  was  $24.3\text{ ng}/\mu\text{l}$  or 94% of the median  $T_0$  concentration (Figure 9). Stability testing of DNA in blood frozen in the PAXgene Blood DNA tube is ongoing. For the most recent test results, see [www.preanalytix.com](http://www.preanalytix.com).

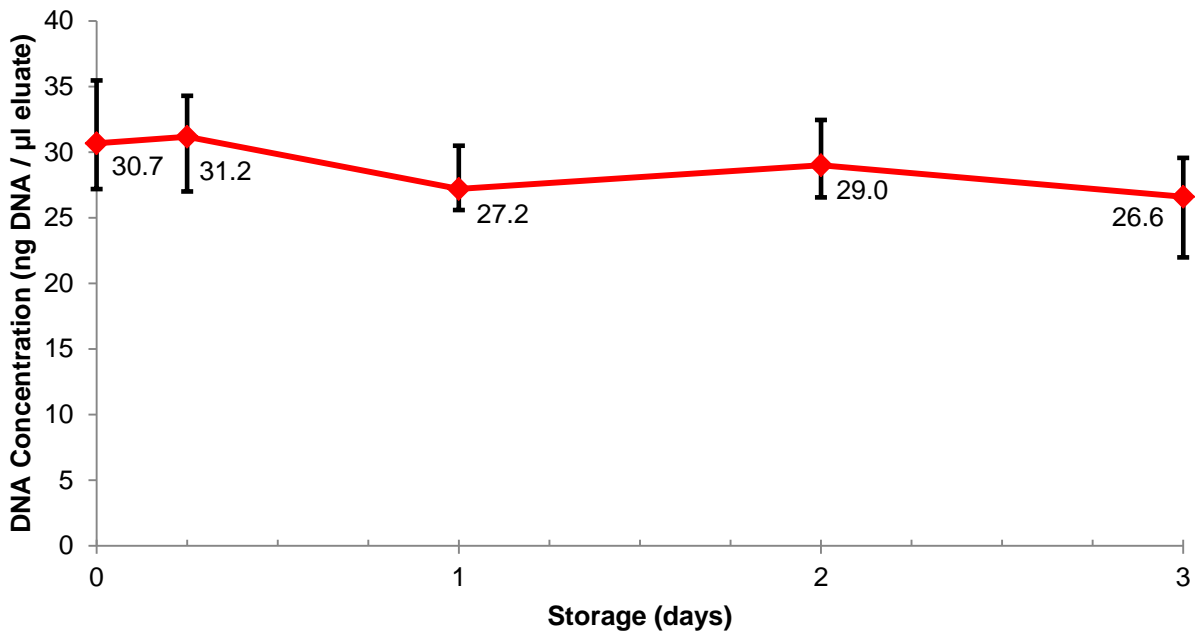


**Figure 5:** Median and 25-75% interquartile ranges for DNA eluate concentrations extracted from blood stored in PAXgene Blood DNA Tubes for 28 days at  $2-8^{\circ}\text{C}$ .

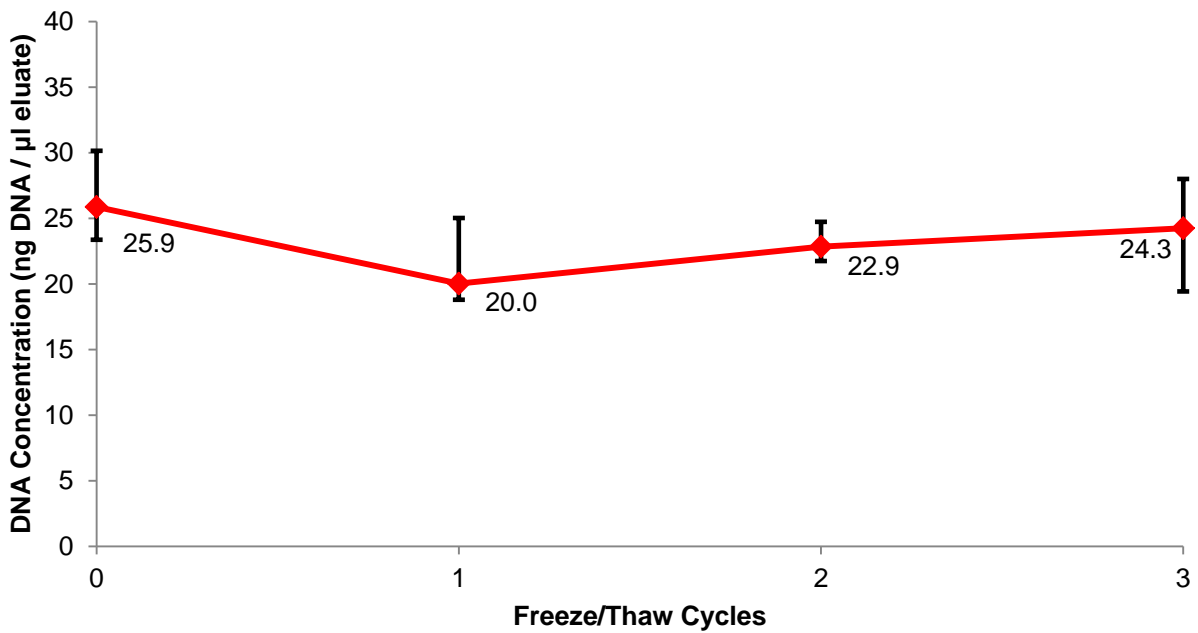


**Figure 6:** Median and 25-75% interquartile ranges for DNA eluate concentrations extracted from blood stored in PAXgene Blood DNA Tubes for 14 days at  $18-25^{\circ}\text{C}$ .

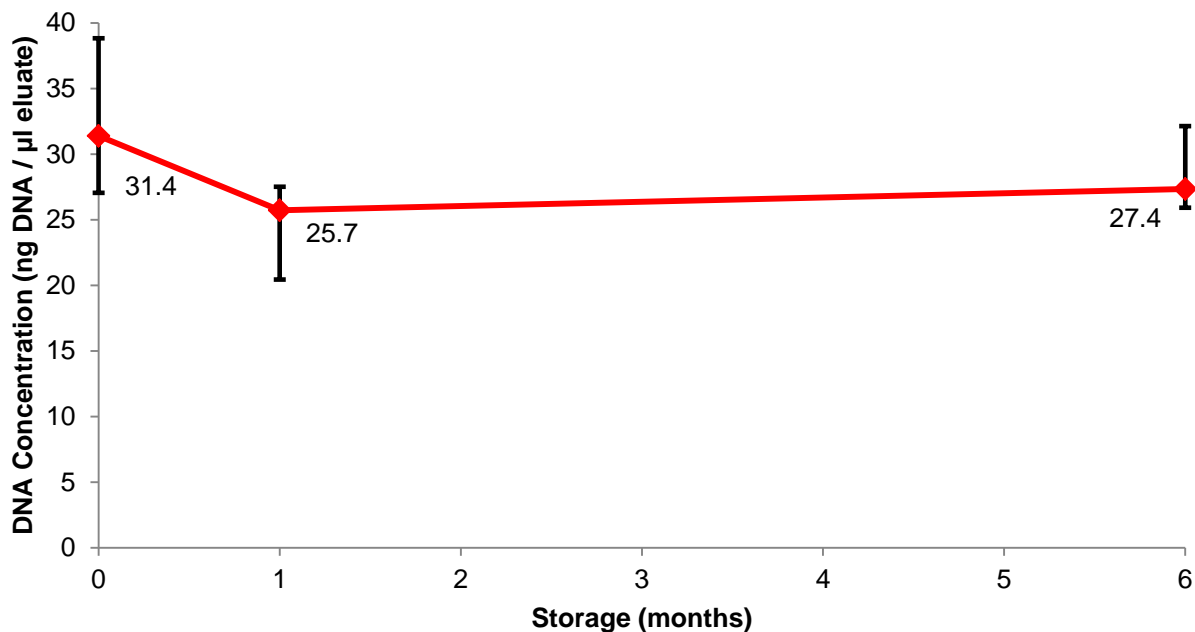




**Figure 7:** Median and 25-75% interquartile ranges for DNA eluate concentrations extracted from blood stored in PAXgene Blood DNA Tubes for 3 days at 35°C.



**Figure 8:** Median and 25-75% interquartile ranges for DNA eluate concentrations extracted from blood stored in PAXgene Blood DNA Tubes subjected to three freeze/thaw cycles.



**Figure 9:** Median and 25-75% interquartile ranges for DNA eluate concentrations extracted from blood stored in PAXgene Blood DNA Tubes for 6 months at -20°C.

## DISCUSSION

We have developed a new blood collection device, the PAXgene Blood DNA Tube, to collect, anticoagulate, stabilize, transport, and store a venous whole blood sample for preparation of high quality DNA for use with molecular diagnostic test methods that require DNA. We have determined that after blood is collected, the DNA remains stable for 28 days at 2-8°C (Figure 5), 14 days at 18-25°C (Figure 6), 3 days at 35°C (Figure 7), after 3 freeze/thaw cycles (Figure 8) or for a minimum of 6 months at -20°C (Figure 9). Whole blood specimens can be frozen and thawed up to three times without appreciable loss of DNA eluate concentration or quality. All DNA concentrations in all eluates obtained from blood specimens, regardless of handling or storage conditions were  $\geq 12.2$  ng/ $\mu$ l and DNA purity as measured by the  $A_{260}/A_{280}$  ratio was between 1.7 and 1.9. Furthermore, all purified DNA samples obtained from blood collected and stored in the PAXgene Blood DNA Tube returned results which were concordant with those of paired EDTA controls when tested in a molecular diagnostic assay. We further demonstrated that the PAXgene Blood DNA Tube is compatible with magnetic bead (QIAasymphony) DNA isolation methods, as well as silica membrane (QIAcube® and QIAamp®) and salting out/precipitation methods (data not shown). Furthermore, blood collected in the PAXgene Blood DNA tube can be stored frozen in situ for at least 6 months with no significant loss of quality or quantity of DNA.

## CONCLUSION

PreAnalytiX has developed the PAXgene Blood DNA Tube specifically for in vitro diagnostic (IVD) DNA testing. We have validated the performance of this tube by evaluating the DNA yield, purity, and performance in a molecular diagnostic assay for multiple transport and storage conditions. Additionally, we have shown that the tube is compatible with various DNA isolation methods including the QIASymphony instrument. Storing and processing whole blood within the conditions tested in these studies assures high quality DNA suitable for molecular diagnostic test methods that require DNA.

The PAXgene Blood DNA Tube is currently not available in the U.S.

Trademarks: PAXgene®, PreAnalytiX® (PreAnalytiX GmbH); QIAamp®, QIAcube®, QIASymphony®, QIAGEN® (QIAGEN Group); BD Vacutainer®, BD® (Becton, Dickinson and Company); EZ Type®, LIFECODES®, Immucor® (Immucor, Inc.); Ac•T diff™, COULTER®, Beckman Coulter® (Beckman Coulter, Inc.); Gen-Probe® (Gen-Probe Incorporated), Hologic® (Hologic, Inc.).

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