PreAnalytiX
A QIAGEN / BD Company

Technical Note

PAXgene® Blood ccfDNA Tube (CE-IVD) Sample Transportation Study (Summer Profile)

The objective of this study was to test the effect of transport on whole blood samples collected into PAXgene Blood ccfDNA Tubes (CE-IVD). In the study, the preanalytical steps including handling, storage, processing and documentation were conducted according to ISO 20186-2:2019 and ISO 20186-3:2019 (Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for venous whole blood – Part 2: Isolated genomic DNA and Part 3: Isolated circulating cell-free DNA from plasma).

Introduction

The PAXgene Blood ccfDNA Tube (PAXgene Tube) is a sterile, single use, plastic, evacuated blood collection tube with a ccfDNA stabilization additive (1.5 ml of liquid additive) and has a nominal blood draw volume of 10 ml. The tube additive is non-crosslinking, free of formaldehyde-releasing substances, stabilizes blood cells, and prevents apoptosis. Blood-filled PAXgene Blood ccfDNA Tubes can be stored for up to 10 days at 2–25°C, up to 7 days at 2–30°C, or up to 3 days at 2–37°C prior to processing. Plasma is separated from the nucleated cellular fraction by a double centrifugation, first for 15 minutes at 1,600–3,000 × *g*, then transfer of the plasma into a secondary tube, followed by centrifugation of the plasma for another 10 minutes at 1,600–3,000 × *g*.

Plasma generated from whole blood collected into PAXgene Blood ccfDNA Tubes (CE-IVD) can be used to process ccfDNA automated with the PreAnalytiX® QIAsymphony® PAXgene Blood ccfDNA Kit (CE-IVD), or manually with the QIAGEN® QIAamp® DSP Circulating Nucleic Acid Kit (CE-IVD). The nucleated cellular fraction or buffy coat remaining after removal of the plasma can be processed for genomic DNA (gDNA) automated with the QIAGEN QIAsymphony DSP DNA Mini and Midi Kits (CE-IVD) or manually with the QIAGEN QIAamp DSP DNA Blood Mini Kit (CE-IVD).

PreAnalytiX developed the PAXgene Blood ccfDNA Tube (CE-IVD) to minimize post-collection changes of circulating, cell-free DNA (ccfDNA) and genomic DNA profiles in whole blood caused by preanalytical workflow variables (**Figure 1**).

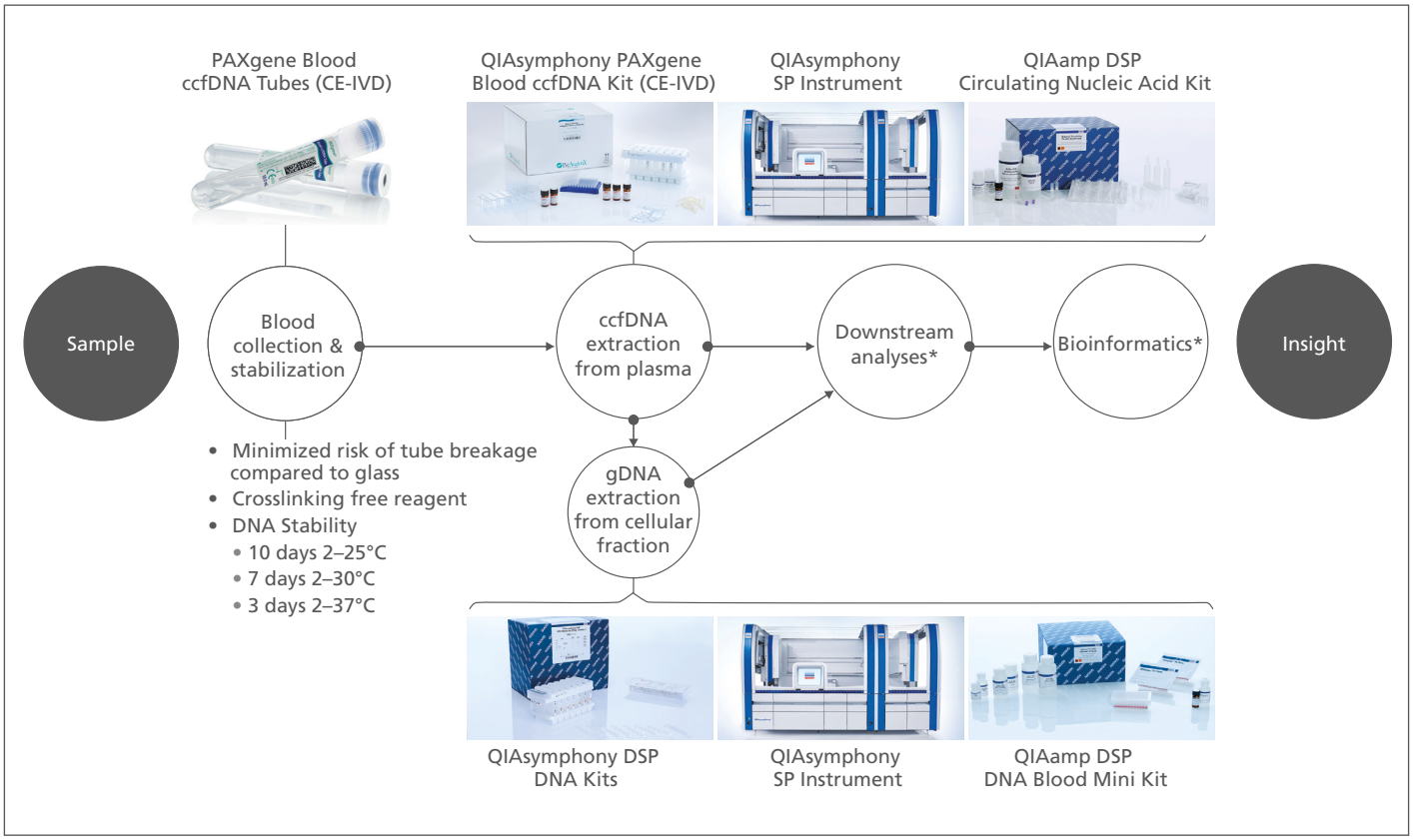

Figure 1. PAXgene Blood ccfDNA workflow (CE-IVD).

The PAXgene Blood ccfDNA Tube (CE-IVD) with the QIAsymphony PAXgene Blood ccfDNA Kit (CE-IVD) and QIAGEN QIAsymphony instrument (CE-IVD) have been verified and validated as an integrated workflow.

Plasma generated from whole blood collected into PAXgene Blood ccfDNA Tubes (CE-IVD) can be used to process ccfDNA automated with the QIAsymphony PAXgene Blood ccfDNA Kit, or manually with the QIAamp DSP Circulating Nucleic Acid Kit. The nucleated cellular fraction or buffy coat remaining after removal of the plasma can be processed for gDNA automated with the QIAsymphony DSP DNA Mini and Midi Kits, or manually with the QIAamp DSP DNA Blood Mini Kit.

*Only when used in combination with products for In Vitro Diagnostic use.

The PAXgene Blood ccfDNA workflow is intended to standardize the preanalytical workflow from blood collection, stabilization, and transport until purification of ccfDNA from plasma and gDNA from the nucleated cellular fraction.

The PAXgene Blood ccfDNA Tube (CE-IVD) is CE marked for In Vitro Diagnostic (IVD) use according to the EU Regulation on in vitro diagnostic medical devices (REGULATION (EU) 2017/746) and as part of IVD development has been intensively tested in various verification and validation studies.

In this technical note, we present the results from a full workflow verification study including all the preanalytical steps from sample collection until the purified analyte is ready for analysis. This included transportation of whole blood by air and ground shipment methods, as well as different sites for collection, plasma processing, and DNA purification.

Study Design

Blood samples from 29 consented, apparently healthy adult subjects were collected into two lots of PAXgene Blood ccfDNA Tubes (CE-IVD) at QIAGEN (Hilden, Germany). Samples from one additional donor were withdrawn from the study due to insufficient fill volume. One tube per lot from each donor was processed immediately (≤ 6 hours) at day 0 (T_0) or stored for 7 days at 30°C, the longest storage duration specified for an elevated temperature above room temperature. One additional tube per lot was transported from QIAGEN to BD (Franklin Lakes, USA) via domestic ground and international air transportation. For the transport, the blood samples were packaged to be IATA-compliant, following DGR (Dangerous Goods Regulation) Packing Instruction 650 for transport of Biological Substances Category B (UN3373). This included a leakproof triple packaging concept with a primary receptacle, a secondary package, and a rigid outer package. No cooling devices were included in the package. To measure temperature fluctuations during transport, a data logger was included.

Upon arrival, samples were processed by a double centrifugation at room temperature with brake set to medium: 15 min 1,900 × *g*, transfer of the plasma into a secondary tube, followed by 10 min 1,900 × *g*, according to the instructions for use for the PAXgene Blood ccfDNA Tube (CE-IVD), to separate the plasma from the nucleated cellular fraction. Both plasma and residual nucleated cellular fraction were frozen at –20°C and sent back to QIAGEN on dry ice (**Figure 2**). Before freezing, to assess the level of hemolysis, a small aliquot of plasma sample was removed from each sample to measure plasma-free hemoglobin (PFHb) on the Beckman Coulter UniCel DxC 680i instrument, according to the instructions of the manufacturer.

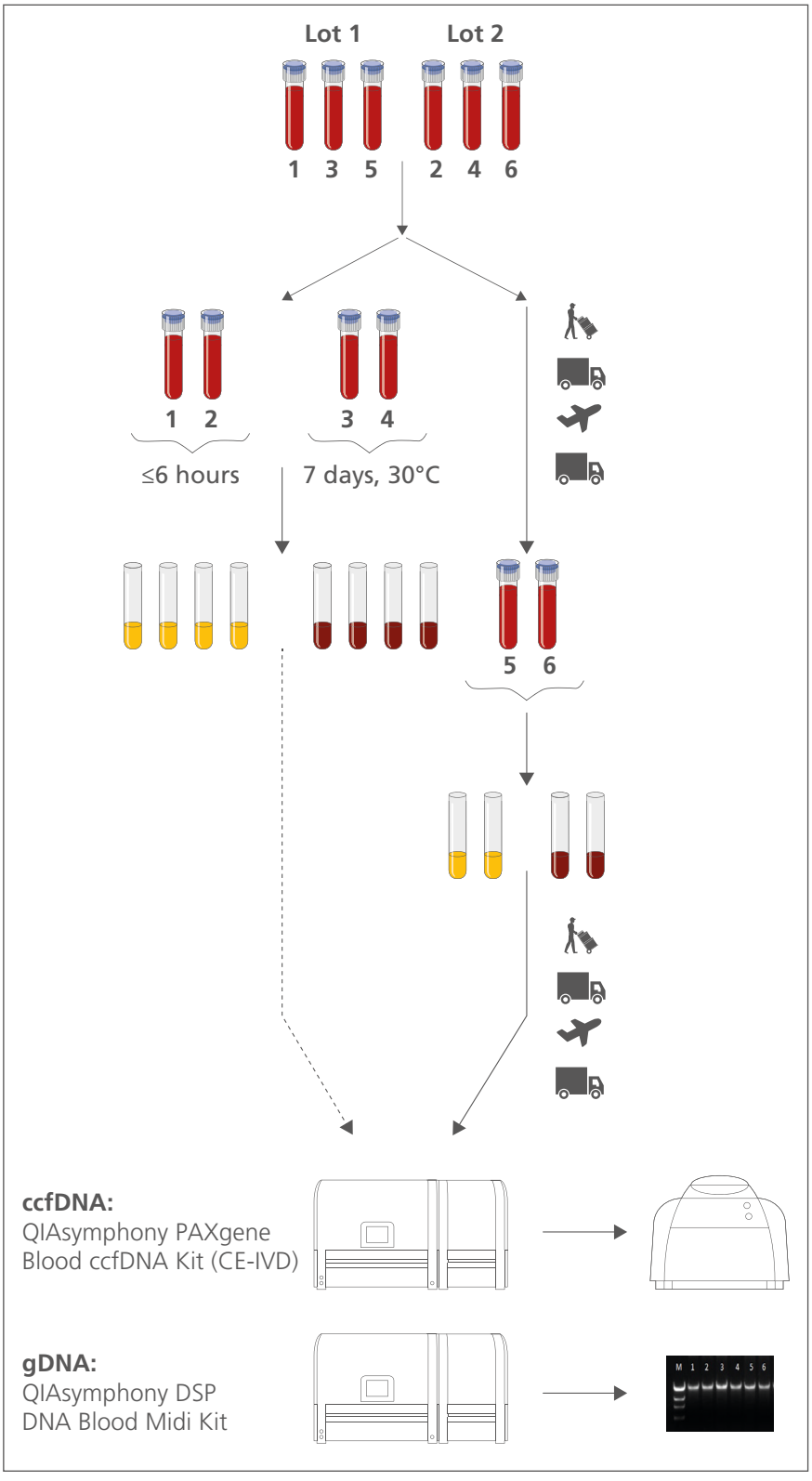

Figure 2. Workflow for the Transport Study.

ccfDNA was extracted from the plasma received at QIAGEN with the QIAsymphony PAXgene Blood ccfDNA Kit (CE-IVD) on the QIAsymphony SP instrument using the 2.4 ml protocol. Relative ccfDNA yield was quantified by a probe-based real-time PCR assay amplifying a 66 bp fragment of the 18S rDNA gene on the QIAGEN Rotor-Gene® Q MDx instrument.

gDNA was extracted from the nucleated cellular fraction received at QIAGEN with the QIAsymphony DSP DNA Midi Kit (CE-IVD) on the QIAsymphony SP instrument using the 400 µl protocol. gDNA yield and purity were measured by spectrophotometry on a NanoDrop™ spectrophotometer (Thermo Fisher Scientific), and gDNA integrity by agarose gel electrophoresis.

Results

The temperature within the package during transport of blood samples was tracked with a data logger (Testo, Germany). It varied between 18 to 35°C, with the highest value occurring during ground transportation in the US from Newark International Airport to the final destination at BD (Franklin Lakes, USA) (**Figure 3**). The temperature range and time between blood collection and processing were within the specifications for the PAXgene Blood ccfDNA Tube (CE-IVD).

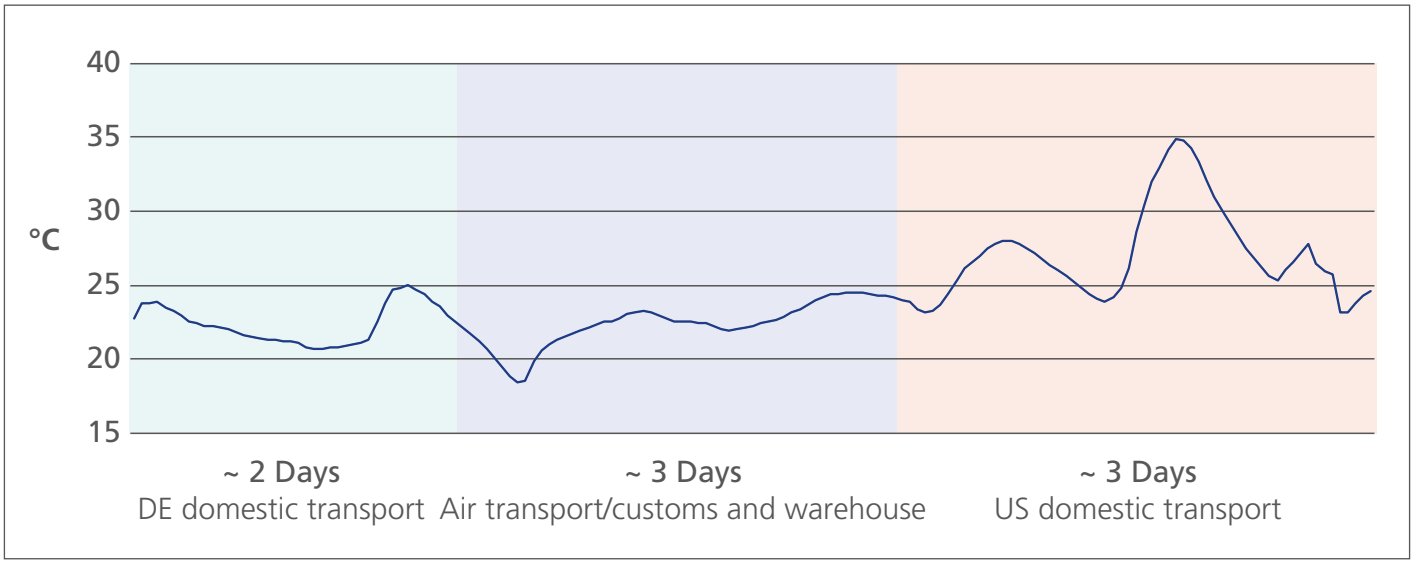

Figure 3. Temperature during Summer Transport of whole blood samples collected into PAXgene Blood ccfDNA Tubes (CE-IVD) from QIAGEN (Hilden, Germany) to BD (Franklin Lakes, USA).

The level of plasma-free hemoglobin in PAXgene Blood ccfDNA Tube (CE-IVD) plasma samples after transportation was higher for some samples compared to the plasma from samples processed directly after blood draw, but comparable to samples stored for 7 days at 30°C, with a median of ~40 mg/dl (**Figure 4 A**). In contrast, as shown previously, unstabilized blood samples from healthy donors collected into K_2EDTA Tubes show increased hemolysis when stored for 7 days at 30°C (**Figure 4 B**).

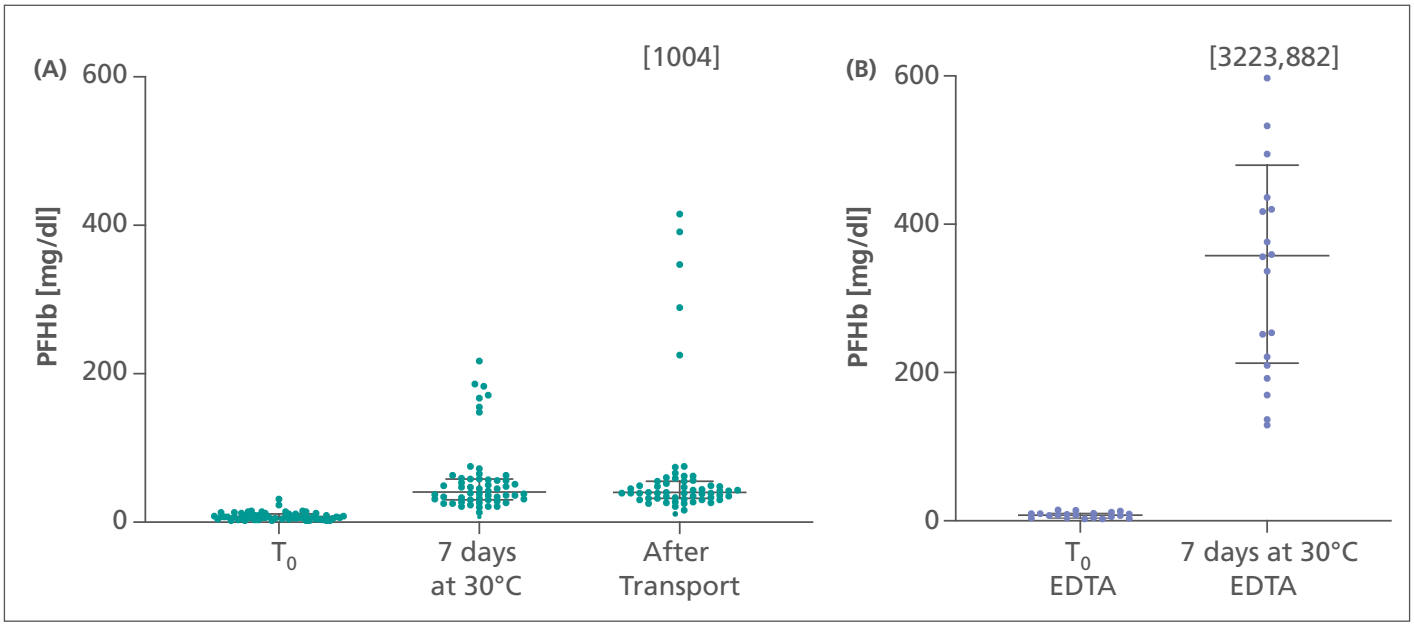

Figure 4. Plasma-free hemoglobin (PFHb) measured on the Beckman Coulter instrument (UniCel DxC 680i).

(**A**) Concentration of PFHb in blood samples from 29 healthy donors collected into two lots of PAXgene Blood ccfDNA Tubes (CE-IVD), processed immediately (T_0), stored for 7 days at 30°C, or transported from QIAGEN (Hilden, Germany) to BD (Franklin Lakes, USA).

(**B**) Concentration of PFHb in blood samples from 20 healthy donors collected into K_2EDTA spray dried tubes processed immediately (T_0), or stored for 7 days at 30°C.Medians and the 25th and 75th percentiles are denoted.

ccfDNA was purified on the fully automated QIAsymphony SP instrument with the QIAsymphony PAXgene Blood ccfDNA Kit (CE-IVD). Yield, measured as copies of 18S rDNA molecules per ml plasma, was slightly increased in plasma from transported samples compared to T_0 samples, but wasn´t significantly different from the corresponding samples stored for 7 days at 30°C (**Figure 5**).

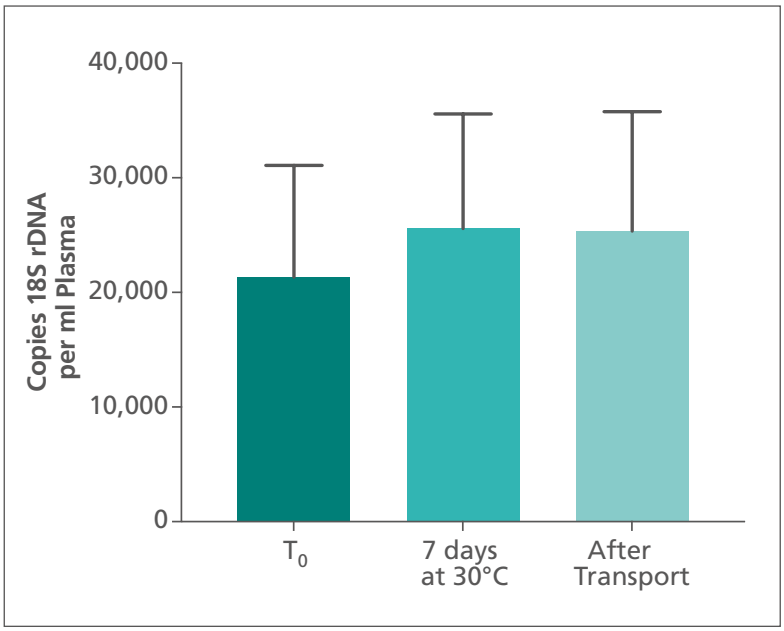

Figure 5. Copies of 18S rDNA per ml plasma in blood samples from 29 healthy donors collected into two lots of PAXgene Blood ccfDNA Tubes (CE-IVD), processed immediately (T_0), stored for 7 days at 30°C or transported from QIAGEN (Hilden, Germany) to BD (Franklin Lakes, USA).

ccfDNA was extracted with the QIAsymphony PAXgene Blood ccfDNA Kit (CE-IVD) on the QIAsymphony SP instrument using the 2.4 ml protocol. Quantification with probe-based validated qPCR assay (target 18S rDNA gene, 66 bp amplicon) on the Rotor-Gene Q instrument. Values are means with standard deviation.

In general, genomic DNA isolated from the nucleated cellular fraction was of high yield, purity, and integrity. A slight drop in yield could be observed between samples stored for 7 days at 30°C or transported compared to samples from day 0 (T_0). With regard to purity and integrity, no significant difference could be observed between all conditions tested (**Figure 6**).

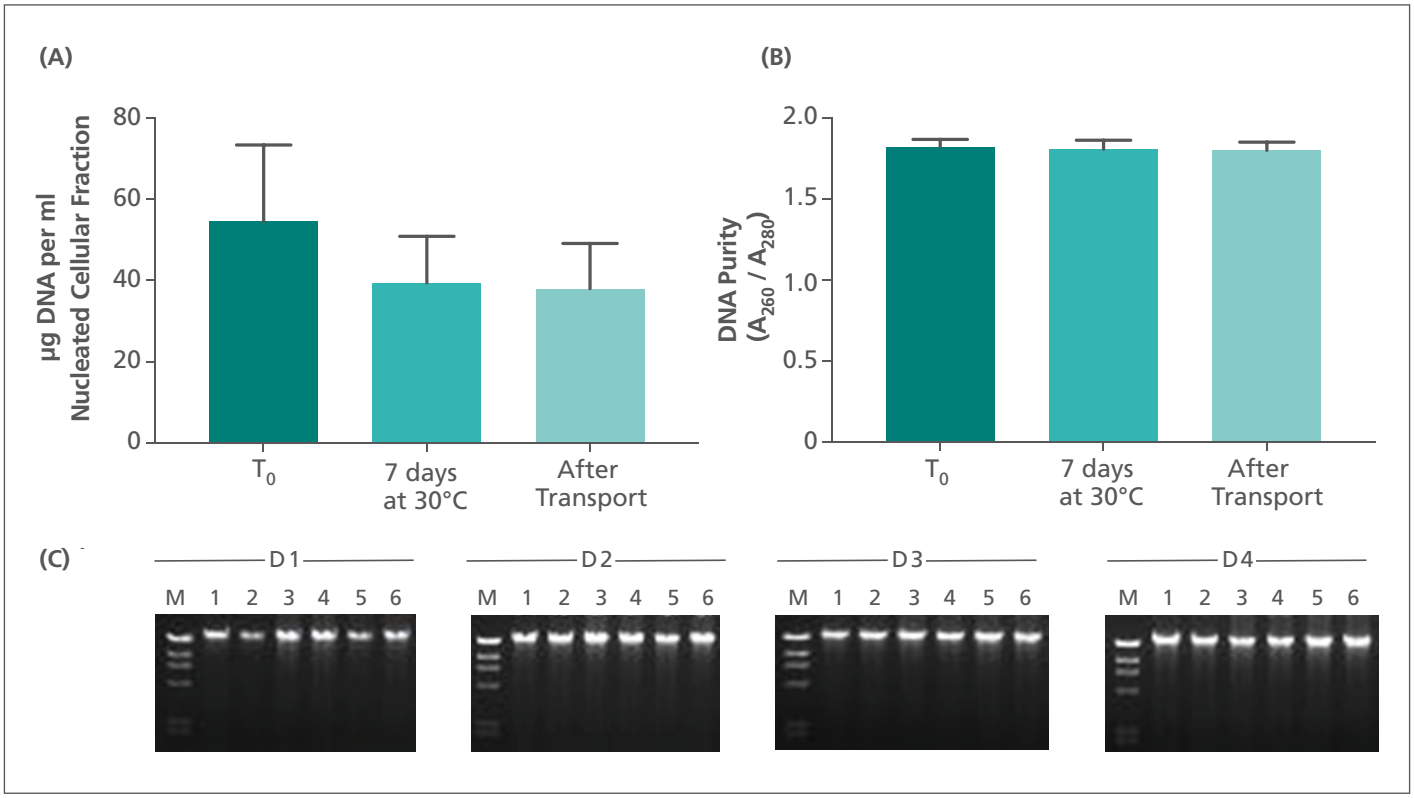

Figure 6. Yield, purity and integrity of genomic DNA from the nucleated cellular fraction of 29 healthy donors collected into two lots of PAXgene Blood ccfDNA Tubes (CE-IVD), processed immediately (T_0), stored for 7 days at 30°C, or transported from QIAGEN (Hilden, Germany) to BD (Franklin Lakes, USA).

DNA was extracted with the QIAsymphony DSP DNA Midi Kit (CE-IVD) on the QIAsymphony SP instrument using the 400 µl protocol. (**A**) gDNA yield in µg DNA per ml nucleated cellular fraction and (**B**) gDNA purity (A_{260} / A_{280}); both yield and purity were measured by spectrophotometry on a NanoDrop spectrophotometer. Values are means with standard deviation. (**C**) DNA integrity using agarose gel electrophoresis is shown for four Donors (D1-D4). Lane 1,2 DNA from T_0; lane 3,4 DNA from 7 days 30°C; lane 5,6 DNA after transport; a Lambda x Hind III marker was loaded into lane "M". The upper band of this marker represents a DNA fragment of 23 kb.

Conclusion

The PAXgene Blood ccfDNA workflow consists of CE-IVD blood collection tube and DNA preparation kits and covers the whole preanalytical workflow from blood collection to DNA purification. Blood samples in the PAXgene Blood ccfDNA Tube (CE-IVD) are stabilized and can be stored or transported within the specified duration and temperature range if immediate centrifugation of the blood collection tube is not possible. Red blood cell breakdown is minimized, which results in reduced hemolysis. Genomic DNA degradation and release from white blood cells is minimized which leads to unchanged concentrations of ccfDNA in plasma as well as consistent high-quality genomic DNA from the nucleated cellular fraction.

For optimal results it is highly recommended to follow the instructions for use for the PAXgene Blood ccfDNA Tube (CE-IVD).

Products used

Product	Catalog No.
PAXgene Blood ccfDNA Tube (CE-IVD) (100)	768165
QIAsymphony PAXgene Blood ccfDNA Kit (CE-IVD) (192)	768566
QIAsymphony DSP DNA Midi Kit (96) (QIAGEN)	937255
QIAsymphony SP instrument (QIAGEN)	9001297
QIAamp DSP Circulating Nucleic Acid Kit (50) (QIAGEN)	61504
Rotor-Gene Q instrument (QIAGEN)	9001550

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The PAXgene Blood ccfDNA Tube (IVD) and QIAsymphony PAXgene Blood ccfDNA Kit (IVD) are distributed by BD and QIAGEN and their distributors and are not available in certain countries including the US. Please visit www.preanalytix.com or contact your local supplier for more details and product availability.

CE 0123

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