
Technical Note

PAXgene[®] Blood ccfDNA Tubes (CE-IVD)* Transport Concept

IATA-compliant packaging of whole blood samples drawn into PAXgene Blood ccfDNA Tubes (CE-IVD)

A transport simulation and functional test conducted according to ISO 20186-3:2019 (*Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for venous whole blood – Part 3: Isolated circulating cell-free DNA from plasma*)

Introduction

PreAnalytiX developed the PAXgene Blood ccfDNA Tube (CE-IVD) to minimize post-collection changes in circulating, cell-free DNA (ccfDNA) profiles or levels and genomic DNA of whole blood caused by preanalytical workflow variables. The device is intended to preserve these biomolecules as well as standardize their collection, stabilization, transport, storage and purification. The PAXgene Blood ccfDNA Tube is compatible with the QIAamp[®] Circulating Nucleic Acid Kit. Blood-filled PAXgene Blood ccfDNA Tubes can be stored for up to 10 days at temperatures up to 25°C, 7 days at temperatures up to 30°C, or 3 days at temperatures up to 37°C. Note: Do not store blood-filled tubes below 2°C.

Blood drawn into a PAXgene Blood ccfDNA Tube should be kept within the temperature range above from collection through to ccfDNA purification. However, temperature control is often not possible throughout transport, especially in warmer areas. Cooling packs may be used, but temperature variations still occur. In this technical note, we present a packaging concept for human whole blood samples drawn into PAXgene Blood ccfDNA Tubes that complies with IATA (International Air Transport Association) regulations (see highlight box). The packaging method maintains the temperature within the specified range and helps stabilize ccfDNA levels. This concept was verified in a transport simulation and functional test.

* The PAXgene Blood ccfDNA Tube (CE-IVD) is intended for in vitro diagnostic use and is only available in some parts of the world outside the United States.

Packaging Instructions for Transport of UN3373, Biological Substance, Category B

Human whole blood samples fall under regulation UN3373, Biological Substance, Category B (<http://www.un3373.com/info/regulations/>). For transportation, the packaging must be fully compliant with IATA DGR (Dangerous Goods Regulation) Packing Instruction 650, which requires labelling instructions and a triple packaging concept including a primary receptacle, a secondary package and a rigid outer package.

The main regulations include:

- The primary receptacle(s) must be leakproof and must not contain more than 1 L;
- The secondary packaging must be leakproof;
- If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them;
- Absorbent material must be placed between the primary receptacle and the secondary packaging. The absorbent material, such as cotton wool, must be in sufficient quantity to absorb the entire contents of the primary receptacle(s) so that any release of the liquid substance will not compromise the integrity of the cushioning material or of the outer packaging;
- The primary receptacle or the secondary packaging must be capable of withstanding, without leakage, an internal pressure of 95 kPa in the range of –40°C to 55°C.
- The outer packaging must not contain more than 4 L.

For more information, please refer to IATA packaging instructions 650, at <https://www.iata.org/whatwedo/cargo/dgr/Documents/packing-instruction-650-DGR56-en.pdf>. Please note that the sender is responsible for complying with labelling and international packaging requirements.

Transport Simulation and Study Design

Blood was drawn from 20 consented, healthy donors, to fill 8 PAXgene Blood ccfDNA Tubes from each. Within 2 hours of blood draw, plasma from 2 tubes per donor was processed as an initial timepoint (T_0) reference. Three packages were prepared, each with 2 tubes per donor, to simulate 3 different high temperature exposures (**Table 1, Figure 1**). Tubes were stored for 1–2 days at room temperatures (15–25°C) before packing. Packages were incubated for either 48 hours at 40°C, 30 hours at 50°C or 24 hours at 60°C, followed by 2–3 days storage at room temperature before ccfDNA extraction. These workflows were intended to simulate short-term storage at the collection site prior to packing and transport, shipment at elevated temperatures, and short-term storage at the analysis site before processing. Timelines included potential storage over a weekend prior to plasma processing and ccfDNA purification (**Table 2**).

Component	Description	Vendor
Primary receptacle	PAXgene Blood ccfDNA Tube (CE-IVD)*	PreAnalytiX
Secondary receptacle	GBox 650	Alex Breuer GmbH
Absorbent sheet	DIM Zell S 5000	Various
Outer packaging	Corrugated box	Various
Cooling pack	Icecatch Solid Insulated	Schaumplast
Expanded polystyrene (EPS) box	EPS box	Schaumplast
Shipping box	Corrugated box	Various

Table 1: Materials used for packaging according to IATA-compliant packaging instructions 650.



Figure 1: IATA-compliant packaging concept. Twenty (20) PAXgene Blood ccfDNA Tubes (CE-IVD) (primary receptacles) filled with blood were placed into a GBox 650 (secondary receptacle). One absorbent sheet was placed below and one on top of the tubes within the GBox. Additional absorbent sheets were wrapped around the tubes **[A]**. To measure the temperature profile, a datalogger was placed within the GBoxes on top of the blood tubes. Two (2) GBoxes were inserted into corrugated boxes (outer packaging) and then placed in the middle of polystyrene box. A cooling pack was placed on each side of the GBoxes **[B]**. Remaining space in the polystyrene box was filled with absorbent sheets and air cushion packaging foil **[C]**. Finally, the polystyrene box was placed into a large corrugated box (shipping box) and labelled appropriately **[D]**.

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	Package 1	Package 2	Package 3
Number of donors	20	20	20
Tubes per donor	2	2	2
Number of tubes	40	40	40
Incubation before packaging	1 day at RT	2 days at RT	1 day at RT
Incubation at elevated temperatures	48 hours at 40°C	30 hours at 50°C	24 hours at 60°C
Incubation before processing	3 days at RT	3 days at RT	4 days at RT

Table 2: Packaging content and transport simulation.

After transport simulation, plasma was processed by double centrifugation according to instructions in the *PAXgene Blood ccfDNA Tube CE-IVD (Instructions for Use) Handbook**, and ccfDNA was purified on the QIASymphony with the QIASymphony PAXgene Blood ccfDNA Kit (RUO)[†] using the protocol **PAXcircDNA_STA_4800** (standard protocol with 4.8 ml plasma). 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 real-time PCR cyclers.

Results

Temperature profiles during transport simulation were measured with a datalogger placed close to the blood collection tubes. After initial incubation at room temperature (RT), the tubes were packed into transport boxes with cooling packs (**Figure 1**) causing the temperature within the secondary receptacles to drop to 10–15°C. During the incubation at 40–60°C, which simulated high-temperature transport conditions, temperatures within the boxes reached up to 37°C and returned to room temperature after the packages were taken out of the incubator (**Figure 2**).

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[†] The QIASymphony PAXgene Blood ccfDNA Kit is for Research Use Only. Not for use in diagnostic procedures.

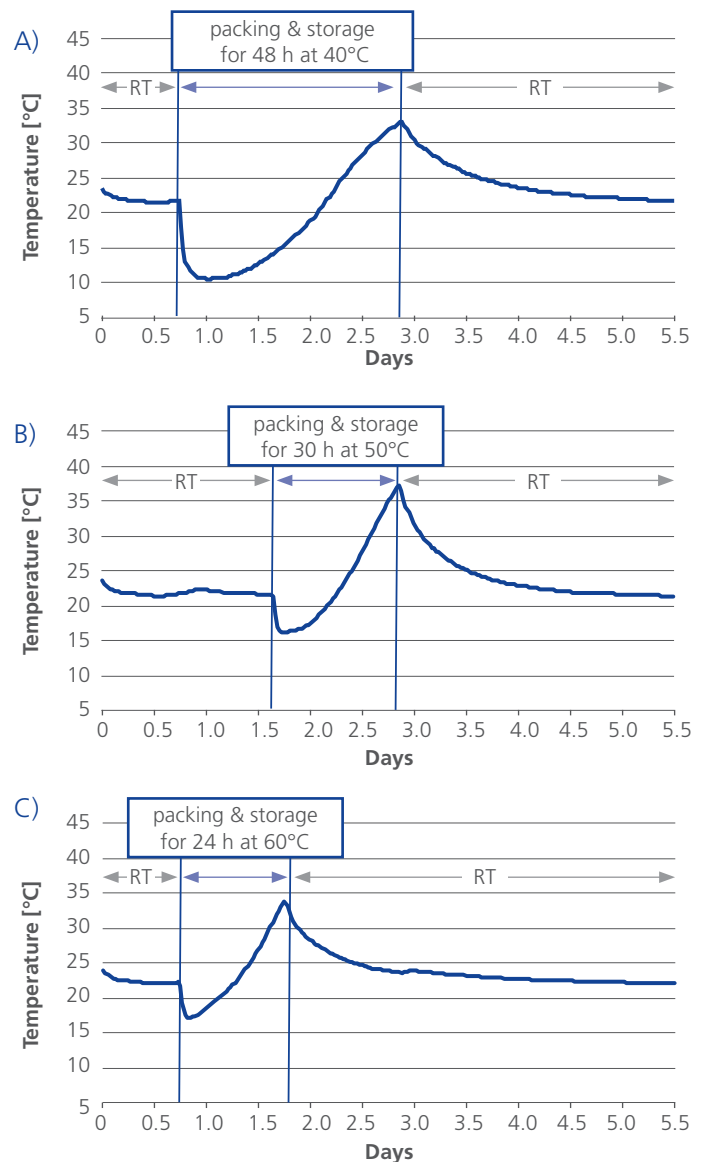


Figure 2: Temperature profiles within the secondary receptacle (GBox 650) containing the blood samples during transport simulation. The secondary receptacles used in the transport simulation were incubated for either 48 h at 40°C [A], 30 h at 50°C [B], or 24 h at 60°C [C].

Relative quantification of ccfDNA purified after the transport simulation compared to samples processed directly after blood collection (T_0) showed high concordance and in situ stability (**Figure 3**).

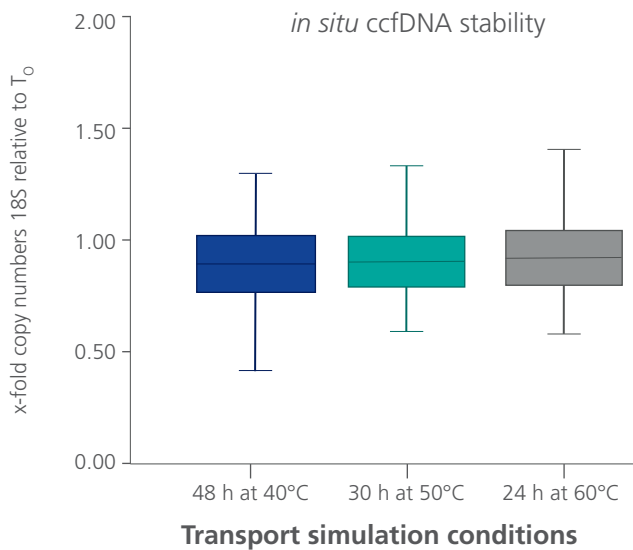


Figure 3: Relative copy number change of 18S rDNA 66bp fragments. Relative quantity of ccfDNA isolated from blood samples of 20 donors (2 samples per donor) after transport simulation involving either 48 h at 40°C, 30 h at 50°C or 24 h at 60°C were compared to matched samples processed directly after blood draw. Change in copy number are shown as box plot with median; n = 40 (a value of 1.0 indicates no change).

Conclusion

Blood samples drawn into PAXgene Blood ccfDNA Tubes (CE-IVD)* can be shipped protected from heat during transport using cooling packs. The PAXgene Blood ccfDNA Tube helps reduce white blood cell apoptosis and the release of genomic DNA into plasma.

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